

PREFACE

On Wednesday, June 23, 1976, the National Institutes of Health issued guidelines to govern NIH-supported research on recombinant DNA molecules. These guidelines, governing research at laboratories of NIH and of its grantees and contractors, delineate stringent safeguards for the conduct of experiments involving the production of recombinant DNA molecules and their insertion into organisms such as bacteria. The NIH guidelines replaced the recommendations from the 1975 Asilomar Conference on Recombinant DNA Molecules, which permitted research under less strict conditions.

Recombinant DNA molecules are formed in the test tube from recombination of segments of deoxyribonucleic acid, the material that determines the hereditary characteristics of all living cells. These techniques, permitting genetic information from quite different organisms to be combined, have a remarkable potential for furthering the understanding of fundamental biochemical processes of lower and higher organisms, and promise to revolutionize molecular biology. They often involve potential hazards, however, which dictate that the research proceed with considerable caution.

Accompanying the guidelines was a document describing in detail the issues which I considered in reaching the decision, as Director of NIH, to release the guidelines developed in the light of many expert judgments. My decision was based on the documents and correspondence contained in the present report. Included is the transcript of a special meeting of the Director's Advisory Committee held at NIH on February 9 and 10, 1976, for the purpose of reviewing proposed courses of action. The guidelines they considered were developed by an NIH Advisory Committee and submitted to the Director of NIH in December 1975.

The meeting of the Director's Advisory Committee afforded an opportunity for the scientific community and the public to comment on the proposed guidelines. Members of the committee represented not only science but such other disciplines as law, ethics, and consumer affairs. The issues raised at that hearing and in the correspondence addressed to me on this matter were carefully reviewed, and my decision, based on this record, examines each of the substantive issues presented. The final guidelines contain a number of revisions based on that analysis.

This report is intended to be the first in a series that will provide the basis for further decisions as the guidelines evolve. On July 7, 1976, NIH published the guidelines in the Federal Register, inviting further public comment. NIH has also undertaken an environmental impact

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assessment in accordance with the National Environmental Policy Act of 1969. The purpose is to assess any environmental effects that could result from research that may be conducted under the guidelines. By these means, continuing opportunity for public review and comment will be provided, to promote sound NIH policy-making in this important research area. Future documents, correspondence, and responses will be considered for publication to provide the public and the scientific community with the basis for NIH policy and decisions.

Donald S. Fredrickson, M.D.
August 1976

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INTRODUCTION

This volume contains significant documents representing events that culminated in the decision of Dr. Donald S. Fredrickson, Director of the National Institutes of Health, to release "Guidelines for Research Involving Recombinant DNA Molecules." The guidelines and the Director's accompanying decision, as published in the Federal Register, are the first documents in this report.

Following those, in chronological order, are documents that explain events leading up to the development and release of the NIH guidelines following extensive public scrutiny and comment.

The Asilomar recommendations and related papers were developed at an international meeting held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. The meeting was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. In attendance were 150 people, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys. The conference reviewed research on recombinant DNA molecules and discussed ways to deal with potential biohazards of the work.

The Asilomar group recommended that experiments on construction of recombinant DNA molecules should proceed, provided that appropriate biological and physical containment is utilized. There were recommendations for matching levels of containment with levels of possible hazard for various types of experiment. Certain experiments judged to pose serious potential dangers were to be prohibited.

The report of the conference's organizing committee, which is included in the present volume, was submitted to the Assembly of Life Sciences of the National Research Council - National Academy of Sciences and approved by its Executive Committee on May 20, 1975. Provisional and summary statements and a list of the Asilomar conference participants appear in the present volume. The summary statement was published in Science, Nature, and Proceedings of the National Academy of Sciences.

The NAS committee that had organized the Asilomar conference at the behest of scientists engaged in recombinant DNA research had also recommended that the Director of NIH establish an advisory committee to evaluate potential hazards of the research and devise guidelines to be followed by investigators. In response, the NIH Recombinant Advisory Committee (formally "NIH Recombinant DNA Molecule Program Advisory Committee") was established in October 1974 to advise the Secretary of HEW, the Assistant Secretary for Health, and the Director of NIH in accomplishing these tasks. The committee proposed guidelines to the Director which are included in this volume.

The several meetings at which the Recombinant Advisory Committee developed its proposed guidelines in 1975 were announced in the Federal Register and were open to the public. At the first meeting, held in San Francisco immediately after the Asilomar conference, it was recommended that NIH use the Asilomar guidelines for research until the committee had an opportunity to elaborate more specific ones. At the second meeting, held on May 12-13 in Bethesda, Maryland, a subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules.

The NIH committee, beginning with the draft prepared by the Hogness subcommittee, developed guidelines at its third meeting, held in July 1975 in Woods Hole, Massachusetts. The Woods Hole draft was circulated. Many critics felt that these guidelines were too lax, others that they were too strict. A new subcommittee chaired by Dr. Elizabeth Kutter was appointed to revise.

A fourth meeting of the NIH committee was held on December 4-5 in La Jolla, California. For this meeting a "variorum edition" had been prepared in which the Hogness, Woods Hole, and Kutter guidelines were compared in detail. The committee voted item-by-item for their preference among the three variations and, in many cases, added new material. The result was the "NIH Proposed Guidelines for Research Involving Recombinant DNA Molecules," which were referred to the NIH Director for final decision.

The Hogness, Woods Hole, and Kutter versions, the variorum edition, and correspondence related to these early drafts are not included in the present volume. These documents, however, are available on request from the National Institutes of Health.

In order to permit further public scrutiny and review, Dr. Fredrickson scheduled a public hearing, notice of which appeared in the Federal Register. He convened for this purpose a special meeting of the Advisory Committee to the Director to review the proposed guidelines. The meeting, open to the public, was held at NIH in Bethesda on February 9-10, 1976. This committee is charged by law to advise the Director, NIH, on matters relating to the broad setting of scientific, technological, and socioeconomic policies in the biomedical sciences. Members are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to committee members, a number of former members and other scientific and public representatives were invited to participate in the special February session.

The transcript of that meeting is contained here. It includes a roster of all members of the committee, all who testified, and all who were invited to attend and present statements. The transcript is a record of the entire day-and-a-half proceeding, and appendixes are included that amplify the record. Following the meeting,

Dr. Fredrickson received extensive correspondence from participants and other parties. The correspondence appears in this volume after the conference proceedings.

From the public hearing and the correspondence, Dr. Fredrickson selected several issues to be reviewed by the Recombinant Advisory Committee at its April meeting. Dr. Fredrickson's letter to the committee members and an accompanying document outlining the issues appear in the present volume. The responses of the Recombinant Advisory Committee were taken into account, and are detailed in the Director's decision paper appearing in the first section of this report.

Special concern was expressed at the public hearing and in related correspondence regarding the application of the guidelines to research conducted outside NIH by investigators other than its grantees or contractors. In response to these suggestions, Dr. Fredrickson held a meeting with representatives of relevant HEW agencies and other departments of the Federal Government. The purpose was to exchange information on recombinant DNA research and to discuss the NIH guidelines. A letter of invitation to the participants, a roster of those attending, and a memorandum summarizing the meeting, held in April 1976, appear in the present volume.

Concerns expressed by commentators about the extension of guidelines to the private sector led Dr. Fredrickson to hold a meeting in June 1976 with representatives of private industry, to provide them with full information about the guidelines and to help determine the present and future interest of industrial laboratories in this type of research. The meeting afforded one of the first opportunities for industry representatives to convene for discussion of this research area. The letter of invitation, a list of the participants, and a brief summary of that meeting are also included.

Because of special interest by congressional staff, a briefing session was held on June 14 to discuss the forthcoming NIH guidelines. Congressional staff invited to attend this briefing session are listed in the present report.

A number of documents are included that relate to the release of the guidelines--the press release, Dr. Fredrickson's opening remarks at a press conference, and selected press articles. In response to public interest in a broad dissemination of the guidelines, a number of documents are included that indicate the extensive distribution nationally and internationally. Also included are representative letters sent to professional organizations soliciting support for the guidelines among their member scientists and to editors of journals requesting editorial endorsement. Lists of the organizations and journal editors receiving letters are enclosed. Finally, a bibliography of selected review articles on recombinant DNA research is provided.

WEDNESDAY, JULY 7, 1976



PART II:

DEPARTMENT OF
HEALTH,
EDUCATION, AND
WELFARE

National Institutes of Health

RECOMBINANT DNA
RESEARCH

Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health RECOMBINANT DNA RESEARCH Guidelines

On Wednesday, June 23, 1976, the Director, National Institutes of Health, with the concurrence of the Secretary of Health, Education, and Welfare, and the Assistant Secretary for Health, issued guidelines that will govern the conduct of NIH supported research on recombinant DNA molecules. The NIH is also undertaking an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969.

The NIH Guidelines establish carefully controlled conditions for the conduct of experiments involving the production of such molecules and their insertion into organisms such as bacteria. These Guidelines replace the recommendations contained in the 1975 *Summary Statement of the Asilomar Conference on Recombinant DNA Molecules*. The latter would have permitted research under less strict conditions than the NIH Guidelines.

The chronology leading to the present Guidelines is described in detail in the NIH Director's decision document that follows. In summary, scientists engaged in this research called, in 1974, for a moratorium on certain kinds of experiments until an international meeting could be convened to consider the potential hazards of recombinant DNA molecules. They also called upon the NIH to establish a committee to provide advice on recombinant DNA technology.

The international meeting was held at the Asilomar Conference Center, Pacific Grove, California, in February 1975. The consensus of this meeting was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate physical and biological barriers. The Asilomar Conference report also made interim assignments of the potential risks associated with different types of experiments. The NIH then assumed responsibility for translating the broadly based Asilomar recommendations into detailed guidelines for research.

The decision by the NIH Director on these Guidelines was reached after extensive scientific and public airing of the issues during the sixteen months which have elapsed since the Asilomar Conference. The issues were discussed at public meetings of the Recombinant DNA Molecule Program Advisory Committee (Recombinant Advisory Committee) and the Advisory Committee to the NIH Director. The Recombinant Advisory Committee extensively debated three different versions of the Guidelines during this period.

The Advisory Committee to the NIH Director, augmented with consultants representing law, ethics, consumer af-

fairs and the environment, was asked to advise as to whether the proposed Guidelines balanced responsibility to protect the public with the potential benefits through the pursuit of new knowledge. The many different points of view expressed at this meeting were taken into consideration in the decision.

The NIH recognizes a special obligation to disseminate information on these guidelines as widely as possible. Accordingly, the Guidelines will be sent to all of the approximately 25,000 NIH grantees and contractors. Major professional societies which represent scientists working in this area will also be asked to endorse the Guidelines. The Guidelines will be sent to medical and scientific journals and editors of these journals will be asked to request that investigators include a description of the physical and biological containment procedures used in any recombinant research they report on. International health and scientific organizations will also receive copies of the guidelines for their review.

Filing of an environmental impact statement will provide opportunity for the scientific community, Federal, State and local agencies and the general public to address the potential benefits and hazards of this research area. In order for there to be further opportunity for public comment and consideration, these guidelines are being offered for general comment in the FEDERAL REGISTER. It must be clearly understood by the reader that the material that follows is *not* proposed rulemaking in the technical sense, but is a document on which early public comment and participation is invited.

Please address any comments on these draft policies and procedures to the Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014. All comments should be received by November 1, 1976.

Additional copies of this notice are available from the Acting Director, Office of Recombinant DNA Activities, National Institute of General Medical Sciences, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

DONALD S. FREDRICKSON,
Director,

NIH National Institutes of Health.

JUNE 25, 1976.

DECISION OF THE DIRECTOR, NATIONAL INSTITUTES OF HEALTH TO RELEASE GUIDELINES FOR RESEARCH ON RECOMBINANT DNA MOLECULES

JUNE 23, 1976.

INTRODUCTION

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 - A. Science Policy.
 - B. Implementation Within the NIH.
 - C. Implementation Beyond the Purview of NIH.
 - D. Environmental Policy.
- II. Methods of Containment (See Guidelines II).
- III. Prohibited Experiments (See Guidelines III, A).
- IV. Permissible Experiments: *E. Coli* K-12 Host-Vector Systems (See Guidelines III, B, 1).

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INTRODUCTION

Today, with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, I am releasing guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules (molecules resulting from the recombination in cell-free systems of segments of deoxyribonucleic acid, the material that determines the hereditary characteristics of all known cells). These guidelines establish carefully controlled conditions for the conduct of experiments involving the insertion of such recombinant genes into organisms, such as bacteria. The chronology leading to the present guidelines and the decision to release them are outlined in this introduction.

In addition to developing these guidelines, NIH has undertaken an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969 (NEPA). The guidelines are being released prior to completion of this assessment. They will replace the current Asilomar guidelines, discussed below, which in many instances allow research to proceed under less strict conditions. Because the NIH guidelines will afford a greater degree of scrutiny and protection, they are being released today, and will be effective while the environmental impact assessment is under way.

Recombinant DNA research brings to the fore certain problems in assessing the potential impact of basic science on society as a whole, including the manner of providing public participation in those assessments. The field of research involved is a rapidly moving one, at the leading edge of biological science. The experiments are extremely technical and complex. Molecular biologists active in this research have means of keeping informed, but even they may fail to keep abreast of the newest developments. It is not surprising that scientists in other fields and the general public have difficulty in understanding advances in recombinant DNA research. Yet public awareness and understanding of this line of investigation is vital.

It was the scientists engaged in recombinant DNA research who called for a moratorium on certain kinds of experiments in order to assess the risks and devise appropriate guidelines. The capability to perform DNA recombinations, and the potential hazards, had become apparent at the Gordon Research Conference on Nucleic Acids in July 1973. Those in attendance voted to send an open letter to Dr. Philip Handler, President of the National Academy of Sciences, and to Dr. John R. Hogness, President of the Institute of Medicine, NAS. The letter, appearing in *Science* 181, 1114, (1973), suggested "that the Academies

[sic] establish a study committee to consider this problem and to recommend specific actions or guidelines, should that seem appropriate."

In response, NAS formed a committee, and its members published another letter in *Science* 185, 303, (1974). Entitled "Potential Biohazards of Recombinant DNA Molecules," the letter proposed:

First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring * * * [certain] experiments * * *.

Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighted * * *.

Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

On October 7, 1974, the NIH Recombinant DNA Molecule Program Advisory Committee (hereafter "Recombinant Advisory Committee") was established to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH, "concerning a program for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants."

The international meeting proposed in the *Science* article (185, 303, 1974) was held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. It was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. One hundred and fifty people attended, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys.

The conference reviewed progress in research on recombinant DNA molecules and discussed ways to deal with the potential biohazards of the work. Participants felt that experiments on construction of recombinant DNA molecules should proceed, provided that appropriate biological and physical containment is utilized. The conference made recommendations for matching levels of containment with levels of possible hazard for various types of experiments. Certain experiments were judged to pose such serious potential dangers that the con-

ference recommended against their being conducted at the present time.

A report on the conference was submitted to the Assembly of Life Sciences, National Research Council, NAS, and approved by its Executive Committee on May 20, 1975. A summary statement of the report was published in *Science* 188, 991 (1975), *Nature* 225, 442, (1975), and the *Proceedings of the National Academy of Sciences* 72, 1981, (1975). The report noted that "in many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard. Until these are established, we urge individual scientists to use the proposals in this document as a guide."

The NIH Recombinant Advisory Committee held its first meeting in San Francisco immediately after the Asilomar conference. It proposed that NIH use the recommendations of the Asilomar conference as guidelines for research until the committee had an opportunity to elaborate more specific guidelines, and that NIH establish a newsletter for informal distribution of information. NIH accepted these recommendations.

At the second meeting, held on May 12-15, 1975, in Bethesda, Maryland, the committee received a report on biohazard-containment facilities in the United States and reviewed a proposed NIH contract program for the construction and testing of microorganisms that would have very limited ability to survive in natural environments and would thereby limit the potential hazards. A subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules, to be discussed at the next meeting.

The NIH committee, beginning with the draft guidelines prepared by the Hogness subcommittee, prepared proposed guidelines for research with recombinant DNA molecules at its third meeting, held on July 18-19, 1975, in Woods Hole, Massachusetts.

Following this meeting, many letters were received which were critical of the guidelines. The majority of critics felt that they were too lax, others that they were too strict. All letters were reviewed by the committee, and a new subcommittee, chaired by Dr. Elizabeth Kutter, was appointed to revise the guidelines.

A fourth committee meeting was held on December 4-5, 1975, in La Jolla, California. For this meeting a "variorum edition" had been prepared, comparing line-for-line the Hogness, Woods Hole, and Kutter guidelines. The committee reviewed these, voting item-by-item for their preference among the three variations and, in many cases, adding new material. The result was the "Proposed Guidelines for Research Involving Recombinant DNA Molecules," which were referred to the Director, NIH, for a final decision in December 1975.

As Director of the National Institutes of Health, I called a special meeting of the Advisory Committee to the Director to review these proposed guidelines. The

meeting was held at NIH, Bethesda, on February 9-10, 1976. The Advisory Committee is charged to advise the Director, NIH, on matters relating to the broad setting—scientific, technological, and socioeconomic—in which the continuing development of the biomedical sciences, education for the health professions, and biomedical communications must take place, and to advise on their implications for NIH policy, program development, resource allocation, and administration. The members of the committee are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to current members of the committee, I invited a number of former committee members as well as other scientific and public representatives to participate in the special February session.

The purpose of the meeting was to seek the committee's advice on the guidelines proposed by the Recombinant Advisory Committee. The Advisory Committee to the Director was asked to determine whether, in their judgment, the guidelines balanced scientific responsibility to the public with scientific freedom to pursue new knowledge.

Public responsibility weighs heavily in this genetic research area. The scientific community must have the public's confidence that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society. A key element in achieving and maintaining this public trust is for the scientific community to ensure an openness and candor in its proceedings. The meetings of the Director's Advisory Committee, the Asilomar group, and the Recombinant Advisory Committee have reflected the intent of science to be an open community in considering the conduct of recombinant DNA experiments. At the Director's Advisory Committee meeting, there was ample opportunity for comment and an airing of the issues, not only by the committee members but by public witnesses as well. All major points of view were broadly represented.

I have been reviewing the guidelines in light of the comments and suggestions made by participants at that meeting, as well as the written comments received afterward. As part of that review I asked the Recombinant Advisory Committee to consider at their meeting of April 1-2, 1976, a number of selected issues raised by the commentators. I have taken those issues and the response of the Recombinant Advisory Committee into account in arriving at my decision on the guidelines. An analysis of the issues and the basis for my decision follow.

I. GENERAL POLICY CONSIDERATIONS

A word of explanation might be interjected at this point as to the nature of the studies in question. Within the past decade, enzymes capable of breaking DNA strands at specific sites and of coupling the broken fragments in new combinations were discovered, thus making possible the insertion of foreign genes into viruses or certain cell particles (plas-

mids). These, in turn, can be used as vectors to introduce the foreign genes into bacteria or into cells of plants or animals in test tubes. Thus transplanted, the genes may impart their hereditary properties to new hosts. These cells can be isolated and cloned—that is, bred into a genetically homogeneous culture. In general, there are two potential uses for the clones so produced: as a tool for studying the transferred genes, and as a new useful agent, say for the production of a scarce hormone.

Recombinant DNA research offers great promise, particularly for improving the understanding and possibly the treatment of various diseases. There is also a potential risk—that microorganisms with transplanted genes may prove hazardous to man or other forms of life. Thus special provisions are necessary for their containment.

All commentators acknowledged the exemplary responsibility of the scientific community in dealing publicly with the potential risks in DNA recombinant research and in calling for a self-imposed moratorium on certain experiments in order to assess potential hazards and devise appropriate guidelines. Most commentators agreed that the process leading to the formulation of the proposed guidelines was a most responsible and responsive one. Suggestions by the commentators on broad policy considerations are presented below. They relate to the science policy aspects of the guidelines, the implementation of the guidelines for NIH grantees and contractors, and the scope and impact of the guidelines nationally and internationally.

A. Science policy considerations

Commentators were divided on how best to steer a course between stifling research through excessive regulation and allowing it to continue with sufficient controls. Several emphasized that the public must have assurance that the controls afford adequate protection against potential hazards. In the views of these commentators, the burden is on the scientific community to show that the danger is minimal and that the benefits are substantial and far outweigh the risks.

Opinion differed on whether the proposed guidelines were an appropriate response to the potential benefits and hazards. Several found the guidelines to so exaggerate safety procedures that inquiry would be unnecessarily retarded, while others found the guidelines weighted toward promoting research. The issue was how to strike a reasonable balance—in fact, a proper policy “bias”—between concerns to “go slow” and those to progress rapidly.

There was strong disagreement about the nature and level of the possible hazards of recombinant DNA research. Several commentators believed that the hazards posed were unique. In their view, the occurrence of an accident or the escape of a vector could initiate an irreversible process, with a potential for creating problems many times greater than those arising from the multitude of

genetic recombinations that occur spontaneously in nature. These commentators stress the moral obligation on the part of the scientific community to do no harm.

Other commentators, however, found the guidelines to be adequate to the hazards posed. In their view, the guidelines struck an appropriate balance so that research could proceed cautiously. Still other commentators found the guidelines too onerous and restrictive in light of the potential benefits of this research for medicine, agriculture, and industry. Some felt that the guidelines are perhaps more stringent than necessary given the available evidence on the likelihood of hazards, but supported them as a compromise that would best serve the scientific community and the public at large. Many commentators urged that the guidelines be adopted as soon as possible to afford more specific direction to this research area.

I understand and appreciate the concerns of those who urge that this research proceed because of the benefits and of those who urge caution because of potential hazards. The guidelines issued today allow the research to go forward in a manner responsive and appropriate to hazards that may be realized in the future.

The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public, or on the environment. The essence of their construction is subdivision of potential experiments by class, decision as to which experiments should be permitted at present, and assignment to these of certain procedures for containment of recombinant organisms.

Containment is defined as physical and biological. Physical containment involves the isolation of the research by procedures which have evolved over many years of experience in laboratories studying infectious microorganisms. P1 containment—the first physical containment level—is that used in most routine bacteriology laboratories. P2 and P3 afford increasing isolation of the research from the environment. P4 represents the most extreme measures used for containing virulent pathogens, and permits no escape of contaminated air, wastes, or untreated materials. Biological containment is the use of vectors or hosts that are crippled by mutation so that the recombinant DNA is incapable of surviving under natural conditions.

The experiments now permitted under the guidelines involve no known additional hazard to the workers or the environment beyond the relatively low risk known to be associated with the source materials. The additional hazards are speculative and therefore not quantifiable. In a real sense they are considerably less certain than are the benefits now clearly derivable from the projected research.

For example, the ability to produce, through “molecular cloning,” relatively large amounts of pure DNA from the chromosomes of any living organism will

have a profound effect in many areas of biology. No other procedure, not even chemical synthesis, can provide pure material corresponding to particular genes. DNA “probes,” prepared from the clones will yield precise evidence on the presence or absence, the organization, and the expression of genes in health and disease.

Potential medical advances were outlined by scientists active in this research area who were present at the meeting of the Director's Advisory Committee. Of enormous importance, for example, is the opportunity to explore the malfunctioning of cells in complicated diseases. Our ability to understand a variety of hereditary defects may be significantly enhanced, with amelioration of their expression a real possibility. There is the potential to elucidate mechanisms in certain cancers, particularly those that might be caused by viruses.

Instead of mere propagation of foreign DNA, the expression of the genes of one organism by the cell machinery of another may alter the new host and open opportunities for manipulating the biological properties of cells. In certain prokaryotes (organisms with a poorly developed nucleus, like bacteria), this exchange of genetic information occurs in nature. Such exchange explains, for instance, an important mechanism for the changing and spreading of resistance to antibiotics in bacteria. Beneficial effects of this mechanism might be the production of medically important compounds for the treatment and control of disease. Examples frequently cited are the production of insulin, growth hormone, specific antibodies, and clotting factors absent in victims of hemophilia.

Aside from the potential medical benefits, a whole host of other applications in science and technology have been envisioned. Examples are the large-scale production of enzymes for industrial use and the development of bacteria that could ingest and destroy oil spills in the sea. Potential benefits in agriculture include the enhancement of nitrogen fixation in certain plants, permitting increased food production.

While the projected research offers the possibility of many benefits, it must proceed only with assurance that potential hazards can be controlled or prevented. Some commentators are concerned that nature may maintain a barrier to the exchange of DNA between prokaryotes and eukaryotes (higher organisms, with a well-formed nucleus)—a barrier that can now be crossed by experimentalists. They further argue that expression of the foreign DNA may alter the host in unpredictable and undesirable ways. Conceivable harm could result if the altered host has a competitive advantage that would foster its survival in some niche within the ecosystem. Other commentators believe that the endless experiments in recombination of DNA which nature has conducted since the beginning of life on the earth, and which have accounted in part for the evolution of species, have most likely involved exchange of DNA between widely disparate species. They

argue that prokaryotes such as bacteria in the intestines of man do exchange DNA with this eukaryotic host and that the failure of the altered prokaryotes to be detected attests to a sharply limited capacity of such recombinants to survive. Thus nature, this argument runs, has already tested the probabilities of harmful recombination and any survivors of such are already in the ecosystem. The fact is that we do not know which of the above-stated propositions is corrected.

The international scientific community, as exemplified by the Asilomar conference and the deliberations attendant upon preparation of the present guidelines, has indicated a desire to proceed with research in a conservative manner. And most of the considerable public commentary on the subject, while urging caution, has also favored proceeding. Three European groups have independently arrived at the opinion that recombinant DNA research should proceed with caution. These are the Working Party on Experimental Manipulation of the Genetic Composition of Micro-Organisms, whose "Ashby Report" was presented to Parliament in the United Kingdom by the Secretary of State for Education and Science in January 1975; the Advisory Committee on Medical Research of the World Health Organization, which issued a press release in July 1975; and the European Molecular Biology Organization Standing Committee on Recombinant DNA, meeting in February 1976.

There is no means for a flat proscription of such research throughout the world community of science. There is also no need to attempt it. It is likely that the evaluation engendered in the preparation and application of these guidelines will lead to beneficial review of some of the containment practices in other work that is not technically defined as recombinant DNA research.

Recombinant DNA research with which these guidelines are concerned involves microorganisms such as bacteria or viruses or cells of higher organisms growing in tissue culture. It is extremely important for the public to be aware that this research is not directed to altering of genes in humans although some of the techniques developed in this research may have relevance if this is attempted in the future.

NIH recognizes its responsibility to conduct and support research designed to determine the extent to which certain potentially harmful effects from recombinant DNA molecules may occur. Among these are experiments, to be conducted under maximum containment, that explore the capability of foreign genes to alter the character of host or vector, rendering it harmful, as through the production of toxic products.

Given the general desire that no rare and unexpected event arising from this research shall cause irreversible damage, it is obvious that merely to establish conservative rules of conduct for one group of scientists is not enough. The precautions must be uniformly and unanimously observed. Second, there

must be full and timely exchange of experiences so that guidelines can be altered on the basis of new knowledge. The guidelines must also be implemented in a manner that protects all concerned—the scientific workers most likely to encounter unexpected hazards and all forms of life within our biosphere. The responsibility of the scientists involved is as inescapable and extreme as is their opportunity to beneficially enrich our understanding.

B. Implementation considerations within the NIH

All the commentators had suggestions concerning the structure and function of decision making as it relates to the principal investigator, the local biohazards committee, the peer review group, and the NIH Recombinant Advisory Committee. These comments and my response on the section of the guidelines relating to roles and responsibilities of investigators, their institutions, and the National Institutes of Health are presented below.

Of considerable concern to all commentators was the process by which NIH would proceed to implement the guidelines. The scientific community generally urged that there be no Federal regulations, while some of the public commentators recommended the regulatory process.

Many who opposed changing the proposed guidelines into Federal regulations expressed concern for flexibility and administrative efficiency, which could best be achieved, in their view, through voluntary compliance. Other commentators, however, believed it imperative to proceed toward regulation. In their view, the guidelines could be implemented for purposes of NIH funding and would govern the conduct of experiments until regulations were in effect. Another commentator who thought regulation would be harmful rather than helpful suggested that if there were to be regulations, they should be along lines similar to those that govern the sale, distribution, use, and disposal of radioisotopes.

The question of how best to proceed now that the guidelines have been released deserves careful attention. I share the concern of those who feel that the guidelines must remain flexible. It is especially important that there be opportunity to change them quickly, based on new information relating to scientific evidence, potential risks, or safety aspects of the research program.

The suggestions for regulation need further attention at this time. The process for regulation not only involves the Director of NIH, but also the Assistant Secretary for Health and the Secretary of Health, Education, and Welfare. These guidelines are being promulgated now in order to afford additional protection to all concerned. Consideration of their conversion to regulations can proceed with continuing review of their content and present and future implications. Meanwhile, the NIH shall continue to provide the opportunity for public comment and participation at least

equivalent to that provided if steps towards regulations were to proceed immediately. The guidelines will be published in the *FEDERAL REGISTER* forthwith to allow for further public comment.

C. Implementation considerations beyond the purview of NIH

Special concern has been expressed by many commentators regarding the application of the guidelines to research outside NIH by investigators other than its grantees or contractors. It has been urged that the guidelines be made applicable to recombinant DNA research conducted or supported by other agencies in HEW and by NSF, ERDA, DoD, and other governmental departments. Most commentators believe that these or similar guidelines should also govern research in the private sector, including industry, voluntary organizations, and foundations. Many feel that experiments conducted in colleges, universities, and even in high schools require some form of monitoring. And finally, all agree that in view of the potential hazards of recombinant DNA research to the biosphere, some form of international understanding on guidelines for the research is essential.

The committee, in the proposed guidelines, has suggested as one means of control that a description of the physical and biological containment procedures practiced in a research project be included in the publication of research results. In the scientific community this can be a powerful force for conformity, and we will undertake to present the recommendation to all appropriate journals. We are also prepared to take steps to disseminate the guidelines widely, and to arrange for a continual flow of information outward concerning the activities of the Recombinant Advisory Committee and the Advisory Committee to the Director, NIH, in the evolution of the guidelines and their implementation.

In response to these suggestions, I have already held a meeting with relevant HEW agencies and with representatives from other departments of the Federal Government. The purpose of the meeting was to exchange information on recombinant DNA research and to discuss the NIH guidelines. It served as an important beginning to address a common concern of these public institutions. A number of the representatives indicated that various departments might very well adopt the guidelines for research conducted both in-house and supported outside. Following up, I have begun preliminary discussions with the Assistant Secretary for Health and the Secretary of HEW, to determine possible methods to ensure adoption of the guidelines by all Federal agencies. Encouraged by these efforts, we held a meeting on June 2 with representatives of industry to provide them with full information about the guidelines and to help determine the present and future interests of industrial laboratories in this type of research. The meeting provided one of the first opportunities for industry representatives to convene for a discussion of this research area, and an industry com-

mittee under the auspices of the Pharmaceutical Manufacturers Association will be formed to review the guidelines for potential application to the drug industry. Further meetings will be scheduled with other groups that have an active interest in recombinant DNA research.

It is my hope that the guidelines will be voluntarily adopted and honored by all who support or conduct such research throughout the United States, and that at least very similar guidelines will obtain throughout the rest of the world. NIH places the highest priority on efforts to inform and to work with international organizations, such as the World Health Organization and the International Council of Scientific Unions, with a view to achieving a consensus on safety standards in this most important research area.

There has been considerable international cooperation and activity in the past, and I expect it to continue in the future. The aforementioned Ashby Report, presented to Parliament in January 1975, describes the advances in knowledge and possible benefits to society of the experiments involving recombinant DNA molecules, and attempts to assess the hazards in these techniques. The Asilomar meeting also had a number of international representatives, as mentioned previously. The European Molecular Biology Organization (EMBO) has been involved in considering guidelines for recombinant DNA research. They have closely followed the activities of NIH, and will thus be encouraged, I believe, to monitor their research with augmented cooperation and coordination. For example, EMBO recently announced plans for a voluntary registry of recombinant DNA research in Europe. Following this EMBO initiative, NIH shall similarly maintain a voluntary registry of investigators and institutions engaged in such research in the United States. Plans for establishing this registry are under way.

D. Environmental policy considerations

A number of commentators urged NIH to consider preparing an environmental impact statement on recombinant DNA research activity. They evoked the possibility that organisms containing recombinant DNA molecules might escape and affect the environment in potentially harmful ways.

I am in full agreement that the potentially harmful effects of this research on the environment should be assessed. As discussed throughout this paper, the guidelines are premised on physical and biological containment to prevent the release or propagation of DNA recombinants outside the laboratory. Deliberate release of organisms into the environment is prohibited. In my view, the stipulated physical and biological containment ensures that this research will proceed with a high degree of safety and precaution. But I recognize the legitimate concern of those urging that an environmental impact assessment be done. In view of this concern and ensuing pub-

lic debate, I have reviewed the appropriateness of such an assessment and have directed that one be undertaken.

The purpose of this assessment will be to review the environmental effects, if any, of research that may be conducted under the guidelines. The assessment will provide further opportunity for all concerned to address the potential benefits and hazards of this most important research activity. I expect a draft of the environmental impact statement should be completed by September 1 for comment by the scientific community, Federal and State agencies, and the general public.

It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research, and alternate approaches to reach those objectives, have been considered. The potential hazards and risks have been analyzed. Alternative approaches have been thoroughly considered, to maximize safety and minimize potential risk. And an elaborate review structure has been created to achieve these safety objectives. From a public policy viewpoint, however, the environmental impact assessment will be yet another review that will provide further opportunity for the public to participate and comment on the conduct of this research.

II. METHODS OF CONTAINMENT

Comments on the containment provisions of the proposed guidelines were directed to the definition of both physical and biological containment and to the safety and effectiveness of the prescribed levels. Several commentators found the concept of physical containment imprecise and too subject to the possibility for human error. Others questioned the concept of biological containment in terms of its safety and purported effectiveness in averting potential hazards. The commentators were divided on which method of containment would provide the most effective and safe system to avoid hazards. Several suggested that each of the physical containment levels be more fully explained.

W. Emmett Barkley, Ph.D., Director of the Office of Research Safety, National Cancer Institute, was asked to review the section on physical containment in light of these comments. Dr. Barkley convened a special committee of safety and health experts, who met to consider not only this section of the guidelines but also the section on the roles and responsibilities of researchers and their institutions. The committee thoroughly reviewed the section on physical containment and recommended a number of changes. The Recombinant Advisory Committee, meeting on April 1-2, 1976, reviewed the recommendations of the Barkley group. These are incorporated, with editorial revisions, in the final version of the guidelines.

The present section on physical containment is directly responsive to those commentators who asked for greater de-

tail and explanation. Although different in detail, the four levels of containment approximate those given by the Center for Disease Control for human etiologic agents and by the National Cancer Institute for oncogenic viruses. For each of the proposed levels, optional items have been excluded, and only those items deemed absolutely necessary for safety are presented. Necessary facilities, practices, and equipment are specified. To give further guidance to investigators and their institutions, a supplement to the guidelines explains more fully safety practices appropriate to recombinant DNA research. And a new section has been added to ensure that shipment of recombinant DNA materials conforms, where appropriate, to the standards, prescribed by the U.S. Public Health Service, the Department of Transportation, and the Civil Aeronautics Board.

The section on physical containment is carefully designed to offer a constructive approach to meeting potential hazards for recombinant experiments at all levels of presumed risk. Certain commentators had suggested that the first level of physical containment (P1) be merged with the second level (P2). This suggestion, however, would tend to apply overly stringent standards for some experiments and might result in a lowering of standards necessary at the second level. I believe the level of control must be consistent with a reasonable estimate of the hazard; and the section on physical containment does provide this consistency. Accordingly, the first and second levels of physical containment remain as separate sections in the guidelines.

Because of the nature and operation of facilities required for experiments to be done at the fourth level of containment (P4), a provision has been included that the NIH shall review such facilities prior to funding them for recombinant DNA studies. The situation merits the special attention of experts who have maximum familiarity with the structure, operation, and potential problems of P4 installations. Several commentators advocated that NIH arrange for sharing of P4 facilities, both in the NIH intramural program and in institutions supported through NIH awards. In response to these suggestions, we are currently reviewing our facilities, including those at the Frederick Cancer Research Center (Fort Detrick), to determine how such a program can best be devised. It is most important that P4 facilities be made available to investigators. It should be noted that incidents of infection by even the most highly infectious and dangerous organisms are extremely infrequent at P4 facilities, and therefore the potential for hazard in certain complex experiments in recombinant DNA research is considerably reduced.

III. PROHIBITED EXPERIMENTS

1. Practically all commentators supported the present prohibition of certain experiments. There were suggestions for a clearer definition of the prohibition of certain experiments where increased antibiotic resistance may result. And it

was urged by some that the prohibition be broadened to include experiments that result in resistance to any antibiotic, irrespective of its use in medicine or agriculture. Consideration of such a suggestion must take into account that antibiotic resistance occurs naturally among bacteria, and that resistance is a valuable marker in the study of microbial genetics in general, and recombinants in particular.

In view of these concerns, however, the Recombinant Advisory Committee was asked to reconsider carefully the prohibition and related sections concerning antibiotic resistance. The committee noted that the prohibition relating to drug resistance was intended to ban those experiments that could compromise drug use in controlling disease agents in veterinary as well as human medicine and this is now clearly stated.

In the draft guidelines there were two statements concerning resistance to drugs which related to experiments with *E. coli*. The statements appeared to allow experiments that would extend the range of resistance of this bacterium to therapeutically useful drugs and disinfectants, and thus seemed to be in conflict with the general prohibition on such research. There are numerous reports in the scientific literature indicating that *E. coli* can acquire resistance to all antibiotics known to act against it. Since *E. coli* acquires resistance naturally, the prohibition directed against increasing resistance does not apply. The ambiguous statements have been deleted from the present guidelines. On the other hand, new language has been inserted in the section dealing with other prokaryote species to set containment levels for permitted experiments.¹

2. The Recombinant Advisory Committee was also asked to clarify whether the prohibition of use of DNA derived from pathogenic organisms (those classified as 3, 4, and 5 by the Center for Disease Control, USPHS) also included the DNA from any host infected with these organisms. The committee explained that this prohibition did extend to experiments with cells known to be so infected. To avoid misunderstanding, the prohibition as now worded includes such cells. In addition, the prohibitions have been extended to include moderate-risk oncogenic viruses, as defined by the National Cancer Institute, and cells known to be infected with them.

3. Two other issues relating to the section on prohibited experiments were raised by Roy Curtiss III, Ph.D., Professor, Department of Microbiology, University of Alabama School of Medicine, Birmingham, who is a member of the Recombinant Advisory Committee. Dr. Curtiss noted that for the class of experiments prohibited on the basis of production of highly toxic substances, only

substances from micro-organisms were cited as examples. He suggested that other examples be included, such as venoms from insects and snakes. The committee approved the suggestion and I concur.

In the proposed guidelines, release of organisms containing recombinant DNA molecules into the environment was prohibited unless a series of controlled tests had been done to leave no reasonable doubt of safety. Dr. Curtiss felt that the guidelines should provide greater specificity for testing and should include some form of review prior to release of the organism. I have decided that the guidelines should, for the present, prohibit any deliberate release of organisms containing recombinant DNA into the environment. With the present limited state of knowledge, it seems highly unlikely that there will be in the near future, any recombinant organism that is universally accepted as being beneficial to introduce into the environment. When the scientific evidence becomes available that the potential benefits of recombinant organisms, particularly for agriculture, are about to be realized, then the guidelines can be altered to meet the needs for release. It is most important that the potential environmental impact of the release be considered.

IV. PERMISSIBLE EXPERIMENTS: *E. COLI*-12 HOST-VECTOR SYSTEMS

The continued use of *E. coli* as a host has drawn considerable comment, including some suggestions that its use be prohibited presently or within a specified time limit. It should be stressed that the use of *E. coli* as detailed in the guidelines is limited to *E. coli* K-12, a strain that has been carried in the laboratory for decades and does not involve the use of any strain of *E. coli* that is freshly isolated from a natural source. *E. coli* K-12 does not usually colonize the normal bowel, even when given in large doses, and exhibits little if any multiplication while passing through the alimentary canal. For years it has been the subject of more intense investigation than any other single organism, and knowledge of its genetic makeup and recombinant behavior exceeds greatly that pertaining to any other organism. I believe that because of this experience, *E. coli* K-12 will provide a host-vector system that is safer than other candidate microorganisms.

NIH recognizes the importance of supporting the development of alternative host-vector systems (such as *B. subtilis*, which has no ecological niche in man) and will encourage such development. It should be noted, however, that for each new host-vector system, the same questions of risk from altered properties attendant upon the presence of recombinant genes will apply as apply to *E. coli*. NIH does not believe it wise to set a time limit on replacement of *E. coli* systems by other organisms.

There were specific suggestions concerning the three levels of biological containment prescribed for use of *E. coli* K-12 host-vectors. Some commentators requested a more detailed explanation of

the adequacy of protection for laboratory personnel with the first level of containment (EK1).² Sections of the guidelines dealing with physical containment and roles and responsibilities now specify the need for safety practices and accident plans.

For the second level of containment (EK2), it is required that a cloned DNA fragment be contained in a host-vector system that has no greater than a 10^{-6} probability of survival in a nonpermissive or natural environment. It was suggested that the selection of this level of biological containment and the appropriate tests for verification be more fully explained in the guidelines. The committee, in responding to a request for further examination of this point, reviewed at considerable length the testing for an EK2 system and recommended certain modifications. We have accepted the committee's new language that better explains testing of survival of a genetic marker carried on the vector, preferably on an inserted NDA fragment.

Possible tests to determine the level of biological containment afforded by these altered host-vector systems are outlined in this section. Because this is such a new area of scientific research and development, however, it is inappropriate to standardize such testing at the present time. Standards will gradually be set as more experience with EK2 host-vector systems is acquired. The committee, for example, during its April 1976 meetings gave its first approval to an EK2 host-vector system. What is necessary is that new and more effective tests be devised by investigators, and this effort is very likely to occur under the present guidelines. For example, one task recognized by the committee is to clarify how survival of the organism and the cloned DNA should be defined in terms of temperature, medium, and other variables.

It is also very important to note here that the stringent requirements set by the committee for EK2 biological containment jeopardize considerably the capacity of such crippled organisms to survive and replicate even under permissive laboratory conditions. More experience will be required to determine whether EK2 containment will permit some lines of important research to be followed.

Several commentators suggested that methods and procedures to confirm an

¹ Specifically, experiments that would extend resistance to therapeutically useful drugs must use P3 physical containment plus a host-vector comparable to EK1, or P2 containment plus a host-vector comparable to EK2.

² The EK1 system presently consists of a battery of different vectors and of *E. coli* K-12 mutants, all of which afford a considerable degree of biological containment. The diversity of vectors and of host mutants in this battery has permitted a wide range of important scientific questions to be attacked. For example, the availability of different vectors with cleavage sites for different restriction endonucleases have increased the kind of DNA segments that can be cloned. By contrast, the first EK2 host-vector systems are only now being considered by the Recombinant Advisory Committee. While NIH is supporting the development of more EK2 host-vector systems, it is not expected that a battery equivalent to that available for the EK1 system will be certified by the Recombinant Advisory Committee in the near future.

EK system at the third level of containment (EK3) be more fully explained. The Recombinant Advisory Committee was asked to consider this suggestion. After considerable discussion the committee declined to define the procedures more fully at this time, because development of an EK3 system is still far enough in the future not to warrant specific testing procedures. Further, it is not clear what tests are best suited. The language, therefore, remains general. The committee, however, is aware of the concerns for a more completely defined system of testing, and has considered the possibility of organizing a symposium for purposes of designating tests. In my view, more fully developed protocols for testing EK3 systems are warranted, and it is necessary that guidelines here be more fully developed before the committee proceeds to certify such a system. In this regard the NIH is prepared through the National Institute of Allergy and Infectious Diseases to support contracts to accomplish this task. We will seek the advice and assistance of the committee to define the scope of necessary work.

These guidelines also include a statement that for the time being no EK2 or EK3 host-vector system will be considered *bona fide* until the Recombinant Advisory Committee has certified it. I share the concern of the commentators that new host-vector systems require the highest quality of scientific review and scrutiny. At this early stage of development, it is most important that the committee provide that scrutiny. Further, I believe that until more experience has been gained, the committee should encourage and the NIH support research that will independently confirm and augment the data on which certification of EK2 host-vector systems are based.

V. CLASSIFICATION OF EXPERIMENTS USING THE *E. COLI* K-12 CONTAINMENT SYSTEMS

The guidelines assign different levels of containment for experiments in which DNA from different sources is to be introduced into an *E. coli* K-12 host-vector system. The variation is based on both facts and assumptions. There are some prokaryotes (bacteria) which constantly exchange DNA with *E. coli*. Here it is assumed that experimental conditions beyond those obtained in careful, routine microbiology laboratories are superfluous, because any exchange experiments have undoubtedly been performed already in nature.

In every instance of artificial recombination, consideration must be given to the possibility that foreign DNA may be translated into protein (expressed), and also to the possibility that normally repressed genes of the host may be expressed and thus change, undesirably, the characteristics of the cell. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely that recombination experiments will create new genetic combinations.

When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and be expressed. Therefore, it is required that experiments involving prokaryotic DNA from a donor that is not known to exchange DNA with *E. coli* in nature be carried out at a higher level of containment. Recombination using prokaryotic DNA from an organism known to be highly pathogenic is prohibited.

There are only limited data available concerning the expression of DNA from higher forms of life (eukaryotes) in *E. coli* (or any other prokaryote). Therefore, the containment prescriptions for experiments inserting eukaryotic DNA into prokaryotes are based on risks having quite uncertain probabilities.

On the assumption that a prokaryote host might translate eukaryotic DNA, it is further presumed that the product of that foreign gene would be most harmful to man if it were an enzyme, hormone, or other protein that was similar (homologous) to proteins already produced by or active in man. An example is a bacterium that could produce insulin. Such a "rogue" bacterium could be of benefit if contained, a nuisance or possibly dangerous if capable of surviving in nature. This is one reason that the higher the phylogenetic order of the eukaryote, the higher the recommended containment, at least until the efficiency of expression of DNA from higher eukaryotes in prokaryotes can be determined.

There is a second, more concrete reason for scaling containment upward as the eukaryote host becomes similar to man. This is the concern that viruses capable of propagating in human tissue, and possibly causing diseases, can contaminate DNA, replicate in prokaryote hosts and infect the experimentalist. Such risks are greatest when total DNA from donor tissue is used in "shotgun" recombinant experiments; it diminishes to much lower levels when pure cloned DNA is used.

The commentators were clearly divided on the classification of containment criteria for different kinds of recombinant DNAs. Many commentators considered the guidelines too stringent and rigid. Others viewed the guidelines in certain instances as too permissive. And still others endorsed the guidelines as sensible and reasonable, affording the public an enormous degree of protection from the speculative risks. Several suggestions were made for the specific classes of experiments, and they follow:

1. Comment on the use of DNA from animals and plants in recombinant experiments varied widely. Some commentators suggested banning the use of DNA from primates, other mammals, and birds. Others suggested that higher levels of containment be used for all such experiments. Still others believed that the guidelines were too strict for experiments of this class. I have carefully reviewed the issues raised by the commentators and the responses of the committee to certain queries concerning use

of animal and plant DNA in these experiments.

In my view, the classification for the use of DNA from primates, other mammals, and birds is appropriate to the potential hazards that might be posed. The physical and biological containment levels are very strict. For example, biological containment levels are at EK2 or EK3, and will effectively preclude experimentation until useful EK2 and EK3 systems are available. EK2 systems are still in the initial stages of development, and the first system was only certified at the most recent meeting of the Recombinant Advisory Committee. An EK3 host-vector system has yet to be tested, and its certification is far enough in the future to place a moratorium on those experiments requiring biological containment at an EK3 level. The physical containment levels of P3 or P4 themselves afford a very high degree of protection. I am satisfied that the guidelines demonstrate the caution and prudence that must govern the conduct of experiments in this category.

The guidelines allow reduced containment levels for primate DNA when it is derived from embryonic tissue or germ-line cells. This is based on evidence that embryonic material is less likely to contain viruses than is tissue from the adult. Obviously, the embryonic tissue must be free of adult tissue, and the present guidelines so indicate.

I have also carefully considered the special concerns arising from the use of DNA from cold-blooded vertebrates and other cold-blooded animals, because several commentators questioned the basis of lower physical and biological containment levels for DNA from these species. The Recombinant Advisory Committee has debated this extensively, and they were asked to do so once again in April.² The committee has now recommended high containment levels (P3+EK2) when the DNA is from a cold-blooded vertebrate known to produce a potent toxin. That recommendation is included in the present guidelines. Where no toxin is involved the committee supported lower

² A committee member, David S. Hogness, Ph. D., Professor, Department of Biochemistry, Stanford University, California, submitted a statement in support of lower containment levels based on current scientific evidence. That evidence is based on certain differences between cold- and warm-blooded vertebrates. One of the criteria used for the evaluation of the relative risk that might be encountered with different levels of shotgun experiment is the degree of sequence homology between the DNA of the given species and that of humans. This criterion is used to estimate the likelihood that segments of DNA from the given species might be integrated into the human genome by recombination; the greater the homology, the greater the likelihood of integration. Studies of sequence homologies indicate that there is a considerable degree of homology between human DNA and DNA from other primates, much less homology between primates and other mammals, and even lower but detectable homology between birds and primates. By contrast, no significant homologies between cold-blooded vertebrates and primates have been detected.

containment levels. The guidelines specify P2+EK2 levels for such work. There was considerable discussion concerning the advisability of recommending lower containment (P2+EK1) when the DNA is isolated from embryonic tissue or germ-line cells from cold-blooded vertebrates. Those supporting lower containment levels argued that the justification for P2+EK2 was the possibility that cold-blooded vertebrates may carry viruses and that the distinction between adult and germ-cell tissue is real. Others argued that, contrary to the situation with primate DNA, viruses are not a central problem with cold-blooded vertebrates and therefore no distinction should be made on the basis of tissue origin. Finally, the committee recommended, on a divided vote (8 to 4), to adopt P2+EK1 when the cold-blooded vertebrate DNA is isolated from embryonic tissue or germ-line cells. Upon reviewing these considerations, I have decided to retain the containment levels for embryonic or germ-line DNA from cold-blooded vertebrates as recommended by the committee.

In April the committee also reviewed, at our request, the classification of experiments where DNA is derived from other cold-blooded animals or lower eukaryotes. Several commentators, for example, had been concerned about the fact that insects are known to carry agents pathogenic to man. In the committee review, it was noted that viruses carried by insects and known to transmit disease to man are RNA rather than DNA viruses and do not reproduce via DNA copied from RNA. In order, however, to make the intent clearer, the guidelines have been rewritten for experiments of this class. New language is inserted to ensure that strict containment levels are employed when the DNA comes from known pathogens or species known to carry them. Further, to reduce the potential hazards, we have also included in the guidelines the requirement that any insect must be grown under laboratory conditions for at least 10 generations prior to its use as a DNA source.

2. As alluded to above, certain commentators expressed concern that when *E. coli* becomes the host of recombinant DNA from prokaryotes with which DNA is not usually exchanged, there is hazard of altered host characteristics resulting from translation of the DNA into functioning proteins. The committee was asked to review the guidelines and take into account this potential hazard. They agreed that the containment levels should be increased for this category of experiment, from P2+EK1 to either P2+EK2 or P3+EK1. That recommendation is included in the present guidelines.

Comments were made concerning that class of experiments in which the recombinant DNA, regardless of source, has been cloned. A clone is a population of cells derived from a single cell and therefore all the cells are presumed to be genetically identical. As outlined in the proposed guidelines, clones could be used at lower containment levels if they had

been rigorously characterized and shown to be free of harmful genes. Several commentators inquired how the characterization was to be performed and the freedom from harmful genes demonstrated. Although the committee acknowledges that these terms are unavoidably vague, they do cite appropriate scientific methods to make relevant determinations. Again, this is a rapidly changing area and more clarity and precision can be expected with experience. Reduced containment requirements for this class of experiment are warranted because of the purified nature of clones. Further, the granting agency must approve the clone before containment conditions can be reduced, thus providing an additional element of review.

4. Another comment was related to the use of DNA from organelles (intracellular elements that contain special groups of genes for particular cell functions). Concern was expressed about the potential contamination of purified organelle DNA with DNA from viruses because of the similarity of their structures. The committee agrees, and the guidelines now specify a requirement, that the organelles be isolated prior to extracting DNA, as a further means of reducing the hazard of viral contamination.

5. Some commentators were troubled about the lowering of containment for that class of experiments involving recombinations with cell DNA segments purified by chemical or physical methods. They asked that procedures for determining the state of purification be more fully detailed and that the Recombinant Advisory Committee certify the purity. There are, however, appropriate techniques, such as gel electrophoresis, with which a purity of 99 percent by mass can be achieved and ascertained. There is no way for the committee to certify these results beyond repeating the experiments themselves. These techniques are well documented and described in the literature. I do not believe it is necessary or feasible for the committee to review each procedure for purification of DNA.

6. Comments were made concerning the use of DNA derived from animal viruses. It was urged that containment levels for this class of experiment be increased. On the basis of my review, I find the containment conditions appropriate to the potential hazard posed. As defined in the guidelines, experiments are to be done at very strict levels of containment and these can be lowered only when the cloned DNA recombinants have been shown to be free of possibly harmful genes by suitable biochemical and biological tests. This also pertains to DNA that is copied from RNA viruses. In no instance are the guidelines more lenient, and in most instances they are more stringent than conditions obtaining in many laboratories where such viruses are studied in non-DNA-recombinant experiments.

VI. CLASSIFICATION OF EXPERIMENTS USING CONTAINMENT SYSTEMS OTHER THAN *E. COLI* K-12

1. No issue with regard to these guidelines raised more comment than the use

of animal viruses as vectors. Of special concern to many commentators was the use of the simian (monkey) virus 40 (hereafter "SV40"). Some suggested a complete ban on the use of this virus; others urged its retention as a vector. SV40 is not known to produce any disease in man, although it can be grown in human cells and on very rare occasions has been isolated from humans. Many humans have received SV40 virus inadvertently in vaccines prepared from virus grown in monkey kidney-cell cultures. An intensive search has been made and is continuing for evidence that SV40 might cause cancer or be otherwise pathogenic for man. At present, it is my view that the extensive knowledge we have of SV40 virus provides us with sufficient sophistication to ensure its safe handling under the conditions developed for its use in the guidelines.

I believe work with SV40 should continue under the most careful conditions, but I do recognize and appreciate the concerns expressed over its possible harmful effects in humans. In light of these concerns, I asked the Recombinant Advisory Committee to review this section of the guidelines. The committee reconsidered the containment conditions for this class of experiments and judged them appropriate to meet the potential hazards.

This class of experiments will proceed under the most careful and stringent conditions. Work with SV40 virus will be done at the maximum level of physical containment (P4). The extraordinary precautions required in a P4 facility lessen the likelihood of a potential hazard from this work. Only defective SV40 virus will be used as vector; that is, the SV40 virus particles that carry the foreign DNA cannot multiply by themselves. When a number of strict conditions are met, this work will be permitted to go on at the third level of containment (P3), which in itself requires care and precision. It should be noted that SV40 virus and its DNA can be efficiently disinfected by Clorox and autoclaving. These are customary procedures for disinfecting glassware and other items used in SV40 animal-cell work.

Some commentators suggested that the containment criteria for experiments using polyoma virus as the vector be strengthened. There is no evidence that polyoma infects humans or replicates to any significant extent in human cells. It holds promise as a vector, as is more fully documented in an appendix to these guidelines.

2. Several commentators found the guidelines inadequate regarding experiments with plant host-vector systems. Because NIH shared these concerns, a group with extensive experience with plants was appointed to review this section. The group met concurrently with

⁴ One member dissented from this position. During the discussion, additional language was recommended (and adopted) to ensure that the defective SV40-virus/helper-virus system, with its inserted non-SV40 DNA segment, does not replicate in human cells with significantly more efficiency than does SV40.

the Recombinant Advisory Committee in April 1976 and made several modifications. The suggested revisions were acceptable to the full committee, and we have included them in the guidelines.

The modifications are responsive to the stated concerns of the commentators. A description of greenhouse facilities is given, and physical containment conditions have been modified to take into account operations with whole plants. On the whole, the respective portions of the guidelines relating to plants are more fully explained and the intent is clarified.

I have also accepted the recommendation of the subcommittee to lower the biological containment level from EK2 to EK1 for experiments in which the DNA from plants is used in conjunction with the *E. coli* K-12 host-vector system, thereby setting containment in this instance at the same level required for experiments with lower-eukaryote DNA.

VII. ROLES AND RESPONSIBILITIES

1. Most commentators had suggestions for the section on the roles and responsibilities of investigators, their local institutions, and NIH. Commentators generally urged openness, candor, and public participation in the process, emphasizing shared responsibility and accountability from the local to the national level. We reviewed that section of the guidelines in light of these comments and have asked the Recombinant Advisory Committee to review certain issues.

It is clear that much of the success of the guidelines will lie in the wisdom with which they are implemented. Because of the importance of this section, especially in terms of safety programs and plans, we have carefully weighed the comments and suggestions made in this regard. NIH has a special responsibility to take a leading role in ensuring that safety programs are part of all recombinant DNA research. Dr. Barkley and a specially convened committee were asked to provide greater detail for safety, accident, and training plans for this section of the guidelines. Based on their recommendations, the section has been extensively rewritten to clarify the respective responsibilities of the principal investigator, the institution (including the institutional biohazards committee), the NIH initial review group (study section), the NIH Recombinant DNA Molecule Program Advisory Committee, and NIH staff.

This section has a definitive administrative framework for assuring that safety is an essential and integrated component of research involving recombinant DNA molecules. The guidelines require investigators to institute, monitor, and evaluate containment and safety practices and procedures. Before research is done, the investigator must have safety and accident plans in place and training exercises for the staff well under way.

Some commentators suggested that the investigator be required to obtain informed consent of laboratory personnel prior to their participation. Rather than rely explicitly on an informed consent document, the guidelines now make the

investigator responsible for advising his program and support staff as to the nature and assessment of the real and potential biohazards. He must explain and provide for any advised or requested precautionary medical policies, vaccinations, or serum collections. Further, an appendix to the guidelines includes detailed explanations for dealing with accidents, as well as instructions for the training of staff in safety and accident procedures.

In response to suggestions for epidemiological monitoring, the guidelines now require the principal investigator to report certain categories of accidents, in writing, to appropriate officials. NIH is investigating procedures for long-term surveillance of workers engaged in recombinant DNA research.

2. A number of comments on the role and responsibilities of the institutional biohazards committee were received. Comments were directed to the structure of the committee, the scope of its responsibility, and the methods for operation. Comments on structure included suggestions that the committee have a broadly based representation, especially in terms of health and safety expertise. Some others suggested NIH require certain classes of representation. In response to these suggestions, the guidelines now recommend membership from a diversity of disciplines relevant to recombinant DNA molecule technology, biological safety, and engineering.

For broader representation beyond the immediate scientific expertise, the guidelines now recommend that local committees should possess, or have available, the competence necessary to determine the acceptability of their findings in terms of applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. The names of and relevant background information on the committee members will be reported to NIH.

In response to suggestions that decisions of the committee be made publicly available, the guidelines now recommend that minutes of the meetings should be kept and made available for public inspection.

Commentators generally approved of the responsibility given to the institutional biohazards committee to serve as a source of advice and reference to the investigator on scientific and safety questions. It was further suggested that the committee's responsibility be broadened in the development, monitoring, and evaluation of safety standards and procedures. In response to these suggestions, the guidelines now indicate that the institutional biohazards committee has the responsibility to certify, and recertify annually, to NIH that the facilities, procedures, practices, training, and expertise of involved personnel have been reviewed and approved. The Recombinant Advisory Committee suggested that examination might be unnecessary for P1 facilities, but we believe that all facilities should be reviewed to emphasize the importance of safety programs.

Some commentators suggested that the guidelines should stipulate that the local

committees be required to determine the containment conditions to be imposed for a given project (which the draft guidelines specifically noted was not their responsibility). The Recombinant Advisory Committee took exception to this suggestion. They urged NIH not to include these conditions as local requirements, arguing among other things that review by the NIH study sections would provide the necessary scrutiny at the national level and assure uniformity of standards in application of the guidelines. I do not believe that NIH should require the local institution to have its biohazards committee assess what containment conditions are required for a given project. On the other hand, the guidelines should not prohibit the local institution from having its biohazards committee perform this function. Accordingly, I have deleted the prohibition that appeared in the proposed guidelines.

Another suggestion was that the local committee ensure that research is carried out in accordance with standards and procedures under the Occupational Safety and Health Act (OSHA). This is an area of importance to the local institutions under Federal and State law, but need not be included as a requirement in the guidelines. NIH will maintain liaison with the Occupational Safety and Health Administration (Department of Labor) to ensure maximum Federal cooperation in this venture.

I would also encourage all institutions, as suggested by several commentators, to review their insurance compensation programs to determine whether their laboratory personnel, in the research area, are covered for injuries.

3. The commentators approved of having the NIH study sections responsible for making an independent evaluation of the classification of the proposed research under the guidelines, along with the customary judgment of the scientific merit of each grant application. This additional element of review will ensure careful attention to potential hazards in the research activity. The study sections will also scrutinize the proposed safeguards. Biological safety expertise shall be available to the study section for consultation and guidance in this regard.

4. Several commentators made suggestions concerning the structure, function, and scope of responsibility of the NIH Recombinant DNA Molecule Program Advisory Committee.

Comments on possible structural mechanisms for decision making included suggestions that there be a scientific and technical committee and a general advisory public policy committee. It was also suggested that the scientific committee include scientists who are not actively engaged in recombinant research, and that the public policy committee have a broad scientific and public representation.

I have carefully reviewed these comments and suggestions. In response, the following structure has been devised. The Recombinant Advisory Committee shall serve as the scientific and technical committee. Its membership shall continue to

include scientists who represent disciplines actively engaged in recombinant DNA research. In my view, it is most important that this committee have the necessary expertise to assure that the guidelines are of the highest scientific quality. The committee has provided this expertise in the past, and it must continue to do so. The committee shall also include members from other scientific disciplines.

It should be noted that the present committee recommended on its own initiative that a nonscientist be appointed. Emmette S. Redford, Ph.D., LL.D., Ashbel Smith Professor of Government and Public Affairs at the Lyndon B. Johnson School of Public Affairs, University of Texas at Austin, serves in that capacity. An ethicist has also been nominated for appointment.

The Advisory Committee to the Director, NIH, shall serve to provide the broader public policy perspectives. This committee, at its meeting on February 9-10, 1976, reviewed the proposed guidelines with the participation of public witnesses, and shall continue to provide such review for future activities of the Recombinant Advisory Committee.

In response to suggestions, the responsibilities of the Recombinant Advisory Committee have been expanded. In addition to reviewing the guidelines for possible modification as scientific evidence warrants, the committee will certify EK2 and EK3 systems. In response to requests by the investigator, local committee, or study section, the committee will also provide evaluation and review in order to advise on levels of required containment, on lowering of requirements when cloned recombinants are to be used, and on questions concerning potential biohazard and adequacy of containment provisions.

Commentators also asked that the committee review ongoing research initiated prior to the implementation of the guidelines. Now that the guidelines are being released, NIH-funded investigators in this field will be asked to give assurance, within a given period, that they will comply. Any investigators who constructed clones under the Asilomar guidelines will be asked to petition NIH for special consideration of their case, if the new guidelines require higher containment than did the Asilomar guidelines. Here the advice of the Recombinant Advisory Committee will be sought.

There were also suggestions that the committee certify chemical purification of recombinant DNA, but as I indicated earlier, these procedures are too well known to require NIH monitoring.

5. In light of comments received, NIH will provide review, through appropriate NIH offices, of data from institutional biohazards committees (including accident reports) and will ensure dissemination of these findings as appropriate. Dr. William Gartland will head the newly created NIH Office of Recombinant DNA Activities for these purposes. In addition, NIH will provide for rapid dissemination of information through its Nucleic Acid Recombinant Scientific

Memoranda (NARSM), distributed by the National Institute for Allergy and Infectious Diseases. NIH will also provide an appropriate mechanism for approving and certifying clones before containment conditions can be lowered.

With these extended modifications, the section of the guidelines dealing with roles and responsibilities now sets forth a more fully developed review structure involving the principal investigator, local biohazards committees, and the Recombinant Advisory Committee, as well as peer review committees. Guidelines now provide extensive opportunity for advice, from the local to the national level. Several levels of review and scrutiny are provided, ensuring the highest standards for scientific merit and conditions for safety.

The Recombinant Advisory Committee in conjunction with the Director's Advisory Committee shall continue to serve as an ongoing forum for examining progress in the technology and safety of recombinant DNA research. Their responsibility, and that of the NIH Director, is to ensure that the guidelines, through modification when called for, reflect the soundest scientific and safety evidence as it accrues in this area. Their task, in a sense, is just beginning.

DONALD S. FREDRICKSON,
Director,
National Institute of Health.

GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

JUNE 1976

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 - B. Institution;
 - C. NIH Initial Review Group (Study Sections);

- D. NIH Recombinant DNA Molecule Program Advisory Committee;
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APPENDICES

- A. Statement on the use of *Bacillus subtilis* in recombinant molecule technology.
- B. Polyoma and SV40 Virus.
- C. Summary of Workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules.
- D. Supplementary Information on Physical Containment (Including Detailed Contents).

I. INTRODUCTION

The purpose of these guidelines is to recommend safeguards for research on recombinant DNA molecules to the National Institutes of Health and to other institutions that support such research. In this context we define recombinant DNAs as molecules that consist of different segments of DNA which have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome.

This is the first attempt to provide a detailed set of guidelines for use by study sections as well as practicing scientists for evaluating research on recombinant DNA molecules. We cannot hope to anticipate all possible lines of imaginative research that are possible with this powerful new methodology. Nevertheless, a considerable volume of written and verbal contributions from scientists in a variety of disciplines has been received. In many instances the views presented to us were contradictory. At present, the hazards may be guessed at, speculated about, or voted upon, but they cannot be known absolutely in the absence of firm experimental data—and, unfortunately, the needed data were, more often than not, unavailable. Our problem then has been to construct guidelines that allow the promise of the methodology to be realized while advocating the considerable caution that is demanded by what we and others view as potential hazards.

In designing these guidelines we have adopted the following principles, which are consistent with the general conclusions that were formulated at the International Conference on Recombinant DNA Molecules held at Asilomar Conference Center, Pacific Grove, California, in February 1975 (3): (i) There are certain experiments for which the assessed potential hazard is so serious that they are not to be attempted at the present time. (ii) The remainder can be undertaken at the present time provided that the experiment is justifiable on the basis that new knowledge or benefits to humankind will accrue that cannot readily be obtained by use of conventional methodology and that appropriate safeguards are incorporated into the design and execution of the experiment. In addition to an insistence on the practice of good microbiological techniques, these safeguards consist of providing both physical

and biological barriers to the dissemination of the potentially hazardous agents. (iii) The level of containment provided by these barriers is to match the estimated potential hazard for each of the different classes of recombinants. For projects in a given class, this level is to be highest at initiation and modified subsequently only if there is a substantiated change in the assessed risk or in the applied methodology. (iv) The guidelines will be subjected to periodic review (at least annually) and modified to reflect improvements in our knowledge of the potential biohazards and of the available safeguards.

In constructing these guidelines it has been necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account existing and anticipated special procedures and information that will allow particular experiments to be carried out under different conditions than indicated here without sacrifice of safety. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that study sections give consideration to such procedures which may allow change in the containment levels recommended here.

It is recommended that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures practiced, to aid and forewarn others who might consider repeating the work.

II. CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities, and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs (4-17). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which either limit the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of

containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the guidelines for research on the different kinds of recombinant DNA (Section III).

A. Standard practices and training. The first principle of containment is a strict adherence to good microbiological practices (4-13). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aseptic techniques and instruction in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

B. Physical containment levels. A variety of combinations (levels) of special practices, equipment, and laboratory installations that provide additional physical barriers can be formed. For example, 31 combinations are listed in "Laboratory Safety at the Center for Disease Control" (4); four levels are associated with the "Classification of Etiologic Agents on the Basis of Hazard" (5), four levels were recommended in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (3); and the National Cancer Institute uses three levels for research on oncogenic viruses (6). We emphasize that these are an aid to, and not a substitute for, good technique. Personnel must be competent in the effective use of all equipment needed for the required containment level as described below. We define only four levels of physical containment here, both because the accuracy with which one can presently assess the biohazards that may result from recombinant DNAs does not warrant a more detailed classification, and because additional flexibility can be obtained by combination of the physical with the biological barriers. Though different in detail, these four levels ($P_1 < P_2 < P_3 < P_4$) approximate those given for human etiologic agents by the Center for Disease Control (i.e., classes 1 through 4; ref. 5), in the Asilomar summary statement (i.e., minimal, low, moderate, and high; ref. 3), and by the National Cancer Institute for oncogenic viruses (i.e., low, moderate, and high; ref. 6), as is indicated by the P-number or adjective in the following

headings. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of hazardous organisms.

We anticipate, and indeed already know of, procedures (14) which enhance physical containment capability in novel ways. For example, miniaturization of screening, handling, and analytical procedures provides substantial containment of a given host-vector system. Thus, such procedures should reduce the need for the standard types of physical containment, and such innovations will be considered by the Recombinant DNA Molecular Program Advisory Committee.

The special practices, equipment and facility installations indicated for each level of physical containment are required for the safety of laboratory workers, other persons, and for the protection of the environment. Optional items have been excluded; only those items deemed absolutely necessary for safety are presented. Thus, the listed requirements present basic safety criteria for each level of physical containment. Other microbiological practices and laboratory techniques which promote safety are to be encouraged. Additional information giving further guidance on physical containment is provided in a supplement to the guidelines (Appendix D).

P1 Level (Minimal). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is a laboratory that possesses no special engineering design features. It is a laboratory commonly used for microorganisms of no or minimal biohazard under ordinary conditions of handling. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available in this laboratory. The laboratory is not separated from the general traffic patterns of the building. Public access is permitted.

The control of biohazards at the P1 level is provided by standard microbiological practices of which the following are examples: (i) Laboratory doors should be kept closed while experiments are in progress. (ii) Work surfaces should be decontaminated daily and following spills of recombinant DNA materials. (iii) Liquid wastes containing recombinant DNA materials should be decontaminated before disposal. (iv) Solid wastes contaminated with recombinant DNA materials should be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. (v) Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed. (vi) Eating, drinking, smoking, and storage of food in the working area should be discouraged. (vii) Facilities to wash hands should be available. (viii) An insect and rodent control program should be provided. (ix) The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

P2 Level (Low). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level is similar in construction and design to the P1 laboratory. The P2 laboratory must have access to an autoclave within the building; it may have a Biological Safety Cabinet.¹ Work which does not produce a considerable aerosol is conducted on the open bench. Although this laboratory is not separated from the general traffic patterns of the building, access to the laboratory is limited when experiments requiring P2 level physical containment are being conducted. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

The P2 laboratory is commonly used for experiments involving microorganisms of low biohazard such as those which have been classified by the Center for Disease Control as Class 2 agents (5).

The following practices shall apply to all experiments requiring P2 level physical containment: (i) Laboratory doors shall be kept closed while experiments are in progress. (ii) Only persons who have been advised of the potential biohazard shall enter the laboratory. (iii) Children under 12 years of age shall not enter the laboratory. (iv) Work surfaces shall be decontaminated daily and immediately following spills of recombinant DNA materials. (v) Liquid wastes of recombinant DNA materials shall be decontaminated before disposal. (vi) Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged materials shall be disposed of by incineration or sterilized before disposal by other methods. Contaminated materials that are to be processed and reused (i.e., glassware) shall be decontaminated before removal from the laboratory. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the working area. (ix) Facilities to wash hands shall be available within the laboratory. Persons handling recombinant DNA materials should be encouraged to wash their hands frequently and when they leave the laboratory. (x) An insect and rodent control program shall be provided. (xi) The use of laboratory gowns, coats, or uniforms is required. Such clothing shall not be worn to the lunch room or outside the building. (xii) Animals not related to the experiment shall not be permitted in the laboratory. (xiii) Biological Safety Cabinets¹ and/or other physical containment equipment shall be used to minimize the hazard of aerosolization of recombinant DNA materials from operations or devices that produce a considerable aerosol (e.g., blender, lyophilizer, sonicator, shaking machine, etc.). (xiv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

P3 Level (Moderate). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas which are open to the general public. Separation is generally achieved by controlled access corridors, air locks, locker rooms or other double-doored facilities which are not available for use by the general public. Access to the laboratory is controlled. Biological Safety Cabinets¹ are available within the controlled laboratory area. An autoclave shall be available within the building and preferably within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination.

Directional air flow is provided within the controlled laboratory area. The ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. The general exhaust air from the laboratory is discharged outdoors and so dispersed to the atmosphere as to prevent reentry into the building. No recirculation of the exhaust air shall be permitted without appropriate treatment.

No work in open vessels involving hosts or vectors containing recombinant DNA molecules requiring P3 physical containment is conducted on the open bench. All such procedures are confined to Biological Safety Cabinets.¹

The following practices shall apply to all experiments requiring P3 level physical containment: (i) The universal biohazard sign is required on all laboratory access doors. Only persons whose entry into the laboratory is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards before entry and they shall comply with posted entry and exit procedures. Children under 12 years of age shall not enter the laboratory. (ii) Laboratory doors shall be kept closed while experiments are in progress. (iii) Biological Safety Cabinets¹ and other physical containment equipment shall be used for all procedures that produce aerosols of recombinant DNA materials (e.g., pipetting, plating, flaming, transfer operations, grinding, blending, drying, sonicating, shaking, etc.). (iv) The work surfaces of Biological Safety Cabinets¹ and other equipment shall be decontaminated following the completion of the experimental activity contained within them. (v) Liquid wastes containing recombinant DNA materials shall be decontaminated before disposal. Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged material shall be sterilized before disposal. Contaminated materials that are to be processed and reused (i.e., glassware) shall be sterilized in the controlled laboratory area or placed in a durable leak-proof container before removal

from the controlled laboratory area. This container shall be sterilized before the materials are processed. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the laboratory. (ix) Facilities to wash hands shall be available within the laboratory. Persons shall wash hands after experiments involving recombinant DNA materials and before leaving the laboratory. (x) In insect and rodent control program shall be provided. (xi) Laboratory clothing that protects street clothing (i.e., long sleeve solid-front or wrap-around gowns, no-button or slipover jackets, etc.) shall be worn in the laboratory. **FRONT-BUTTON LABORATORY COATS ARE UNSUITABLE.** Gloves shall be worn when handling recombinant DNA materials. Provision for laboratory shoes is recommended. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry. (xii) Raincoats, overcoats, topcoats, coats, hats, caps, and such street outerwear shall not be kept in the laboratory.

(xiii) Animals and plants not related to the experiment shall not be permitted in the laboratory. (xiv) Vacuum lines shall be protected by filters and liquid traps. (xv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xvi) If experiments of lesser biohazard potential are to be conducted in the same laboratory concurrently with experiments requiring P3 level physical containment they shall be conducted only in accordance with all P3 level requirements. (xvii) Experiments requiring P3 level physical containment can be conducted in laboratories where the directional air flow and general exhaust air conditions described above cannot be achieved, provided that this work is conducted in accordance with all other requirements listed and is contained in a Biological Safety Cabinet¹ with attached glove ports and gloves. All materials before removal from the Biological Safety Cabinet¹ shall be sterilized or transferred to a non-breakable, sealed container, which is then removed from the cabinet through a chemical decontamination tank, autoclave, ultraviolet air lock, or after the entire cabinet has been decontaminated.

P4 Level (High). Experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease. The facility is either a separate building or it is a controlled area, within a building, which is completely isolated from all other areas of the building. Access to the facility is under strict control. A specific facility operations manual is available. Class III Biological Safety Cabinets¹ are available within work areas of the facility.

A P4 facility has engineering features which are designed to prevent the escape of microorganisms to the environment (14, 15, 16, 17). These features include:

¹ Footnotes at end of article.

(i) Monolithic walls, floods, and ceilings in which all penetrations such as for air ducts, electrical conduits, and utility pipes are sealed to assure the physical isolation of the work area and to facilitate housekeeping and space decontamination; (ii) air locks through which supplies and materials can be brought safely into the facility; (iii) contiguous clothing change and shower rooms through which personnel enter into and exit from the facility; (iv) double-door autoclaves to sterilize and safely remove wastes and other materials from the facility; (v) a biowaste treatment system to sterilize liquid effluents if facility drains are installed; (vi) a separate ventilation system which maintains negative air pressures and directional air flow within the facility; and (vii) a treatment system to decontaminate exhaust air before it is dispersed to the atmosphere. A central vacuum utility system is not encouraged; if one is installed, each branch line leading to a laboratory shall be protected by a high efficiency particulate air filter.

The following practices shall apply to all experiments requiring P4 level physical containment: (i) The universal biohazard sign is required on all facility access doors and all interior doors to individual laboratory rooms where experiments are conducted. Only persons whose entry into the facility or individual laboratory rooms is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards and instructed as to the appropriate safeguards to ensure their safety before entry. Such persons shall comply with the instructions and all other posted entry and exit procedures. Under no condition shall children under 15 years of age be allowed entry. (ii) Personnel shall enter into and exit from the facility only through the clothing change and shower rooms. Personnel shall shower at each exit from the facility. The air locks shall not be used for personnel entry or exit except for emergencies. (iii) Street clothing shall be removed in the outer facility side of the clothing change area and kept there. Complete laboratory clothing including undergarments, pants and shirts or jumpsuits, shoes, head cover, and gloves shall be provided and used by all persons who enter into the facility. Upon exit, this clothing shall be stored in lockers provided for this purpose or discarded into collection hampers before personnel enter into the shower area. (iv) Supplies and materials to be taken into the facility shall be placed in an entry air lock. After the outer door (opening to the corridor outside of facility) has been secured, personnel occupying the facility shall retrieve the supplies and materials by opening the interior air lock door. This door shall be secured after supplies and materials are brought into the facility. (v) Doors to laboratory rooms within the facility shall be kept closed while experiments are in progress. (vi) Experimental procedures requiring P4 level physical containment shall be confined to Class III Biological Safety Cabinets.¹ All materials, before

removal from these cabinets, shall be sterilized or transferred to a non-breakable sealed container, which is then removed from the system through a chemical decontaminated tank, autoclave, or after the entire system has been decontaminated.

(vii) No materials shall be removed from the facility unless they have been sterilized or decontaminated in a manner to prevent the release of agents requiring P4 physical containment. All wastes and other materials and equipment not damaged by high temperature or steam shall be sterilized in the double-door autoclave. Biological materials to be removed from the facility shall be transferred to a non-breakable sealed container which is then removed from the facility through a chemical decontamination tank or a chamber designed for gas sterilization. Other materials which may be damaged by temperature or steam shall be sterilized by gaseous or vapor methods in an air lock or chamber designed for this purpose. (viii) Eating, drinking, smoking, and storage of food are not permitted in the facility. Foot-operated water fountains located in the facility corridors are permitted. Separate potable water piping shall be provided for these water fountains. (ix) Facilities to wash hands shall be available within the facility. Persons shall wash hands after experiments. (x) An insect and rodent control program shall be provided. (xi) Animals and plants not related to the experiment shall not be permitted in the facility. (xii) If a central vacuum system is provided, each vacuum outlet shall be protected by a filter and liquid trap in addition to the branch line HEPA filter mentioned above. (xiii) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xiv) If experiments of lesser biohazard potential are to be conducted in the facility concurrently with experiments requiring P4 level containment, they shall be confined in Class I or Class II Biological Safety Cabinets¹ or isolated by other physical containment equipment. Work surfaces of Biological Safety Cabinets¹ and other equipment shall be decontaminated following the completion of the experimental activity contained within them. Mechanical pipetting devices shall be used. All other practices listed above with the exception of (vi) shall apply.

C. *Shipment.* To protect product, personnel, and the environment, all recombinant DNA material will be shipped in containers that meet the requirements issued by the U.S. Public Health Service (Section 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (Section 173.387(b) of Part 173, Title 49, Code of Federal Regulations) and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6-D) for shipment of etiologic agents. Labeling requirements specified in these Federal regulations and tariffs will apply to all viable recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in

paragraph (c) of 42 CFR 72.25. Additional information on packing and shipping is given in a supplement to the guidelines (Appendix D, part X).

D. *Biological containment levels.* Biological barriers are specific to each host-vector system. Hence the criteria for this mechanism of containment cannot be generalized to the same extent as for physical containment. This is particularly true at the present time when our experience with existing host-vector systems and our predictive knowledge about projected systems are sparse. The classification of experiments with recombinant DNAs that is necessary for the construction of the experimental guidelines (Section III) can be accomplished with least confusion if we use the host-vector system as the primary element and the source of the inserted DNA as the secondary element in the classification. It is therefore convenient to specify the nature of the biological containment under host-vector headings such as those given below for *Escherichia coli* K-12.

III. EXPERIMENTAL GUIDELINES

A general rule that, though obvious, deserves statement is that the level of containment required for any experiment on DNA recombinants shall never be less than that required for the most hazardous component used to construct and clone the recombinant DNA (i.e., vector, host, and inserted DNA). In most cases the level of containment will be greater, particularly when the recombinant DNA is formed from species that ordinarily do not exchange genetic information. Handling the purified DNA will generally require less stringent precautions than will propagating the DNA. However, the DNA itself should be handled at least as carefully as one would handle the most dangerous of the DNAs used to make it.

The above rule by itself effectively precludes certain experiments—namely, those in which one of the components is in Class 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (5), as these are excluded from the United States by law and USDA administrative policy. There are additional experiments which may engender such serious biohazards that they are not to be performed at this time. These are considered prior to presentation of the containment guidelines for permissible experiments.

A. *Experiments that are not to be performed.* We recognize that it can be argued that certain of the recombinants placed in this category could be adequately contained at this time. Nonetheless, our estimates of the possible dangers that may ensue if that containment fails are of such a magnitude that we consider it the wisest policy to at least defer experiments on these recombinant DNAs until there is more information to accurately assess that danger and to allow the construction of more effective biological barriers. In this respect, these guidelines are more stringent than those initially recommended (1).

The following experiments are not to be initiated at the present time: (i) Clon-

¹ See footnotes at end of article.

ing of recombinant DNAs derived from the pathogenic organisms in Classes 3, 4, and 5 of "Classification of Etiologic Agents on the Basis of Hazard" (5), or oncogenic viruses classified by NCI as moderate risk (6), or cells known to be infected with such agents, regardless of the host-vector system used. (ii) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of potent toxins (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.). (iii) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase virulence and host range. (iv) Deliberate release into the environment of any organism containing a recombinant DNA molecule. (v) Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

In addition, at this time large-scale experiments (e.g., more than 10 liters of culture) with recombinant DNAs known to make harmful products are not to be carried out. We differentiate between small- and large-scale experiments with such DNAs because the probability of escape from containment barriers normally increases with increasing scale. However, specific experiments in this category that are of direct societal benefit may be excepted from this rule if special biological containment precautions and equipment designed for large-scale operations are used, and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee of NIH.

B. Containment guidelines for permissible experiments. It is anticipated that most recombinant DNA experiments initiated before these guidelines are next reviewed (i.e., within the year) will employ *E. coli* K-12 host-vector systems. These are also the systems for which we have the most experience and knowledge regarding the effectiveness of the containment provided by existing hosts and vectors necessary for the construction of more effective biological barriers.

For these reasons, *E. coli* K-12 appears to be the system of choice at this time, although we have carefully considered arguments that many of the potential dangers are compounded by using an organism as intimately connected with a man as is *E. coli*. Thus, while proceeding cautiously with *E. coli*, serious efforts should be made toward developing alternate host-vector systems; this subject is discussed in considerable detail in Appendix A.

We therefore consider DNA recombinants in *E. coli* K-12 before proceeding to other host-vector systems.

1. Biological containment criteria using *E. coli* K-12 host-vectors—EK1 host-vectors. These are host-vector systems that can be estimated to already provide a moderate level of containment, and include most of the presently available systems. The host is always *E. coli* K-12, and the vectors include nonconjugative plasmids [e.g., pSC101, ColE1 or deriva-

tives thereof (19-26)] and variants of bacteriophage λ (27-29).

The *E. coli* K-12 nonconjugative plasmid system is taken as an example to illustrate the approximate level of containment referred to here. The available data from experiments involving the feeding of bacteria to humans and calves (30-32) indicate that *E. coli* K-12 did not usually colonize the normal bowel, and exhibited little, if any, multiplication while passing through the alimentary tract even after feeding high doses (i.e., 10^9 to 10^{10} bacteria per human or calf). However, general extrapolation of these results may not be warranted because the implantation of bacteria into the intestinal tract depends on a number of parameters, such as the nature of the intestinal flora present in a given individual and the physiological state of the inoculum. Moreover, since viable *E. coli* K-12 can be found in the feces after humans are fed 10^7 bacteria in broth (30) or 3×10^4 bacteria protected by suspension in milk (31), transductional and conjugational transfer of the plasmid vectors from *E. coli* K-12 to resident bacteria in the fecal matter before and after excretion must also be considered.

The nonconjugative plasmid vectors cannot promote their own transfers, but require the presence of a conjugative plasmid for mobilization and transfer to other bacteria. When present in the same cell with derepressed conjugative plasmids such as F⁺ or R1d⁺19, the nonconjugative ColE1, ColE1-*trp* and pSC101 plasmids are transferred to suitable recipient strains under ideal laboratory conditions at frequencies of about $0.5 \cdot 10^{-4}$ to 10^{-5} , and 10^{-6} per donor cell, respectively. These frequencies are reduced by another factor of 10^3 to 10^4 if the conjugative plasmid employed is repressed with respect to expression of donor fertility.

The experimental transfer system which most closely resembles nonconjugative plasmid transfer in nature is a triparental mating. In such matings, the bacterial cell possessing the nonconjugative plasmid must first acquire a conjugative plasmid from another cell before it can transfer the nonconjugative plasmid to a secondary recipient. With ColE1, the frequencies of transfer are 10^{-2} and 10^{-4} to 10^{-6} when using conjugative plasmid donors possessing derepressed and repressed plasmids, respectively. Mobilization of ColE1-*trp* and pSC101 under similar laboratory conditions is so low as to be usually undetectable (33). Since most conjugative plasmids in nature are repressed for expression of donor fertility, the frequency at which nonconjugative plasmids are mobilized and transferred by this sequence of events *in vivo* is difficult to estimate. However, in calves fed on an antibiotic-supplemented diet, it has been estimated that such triparental nonconjugative R plasmid transfer occurs at frequencies of no more than 10^{-10} to 10^{-12} per 24 hours per calf (32). In terms of considering other means for plasmid transmission in nature, it should be noted that transduction does operate *in*

vivo for *Staphylococcus aureus* (34) and probably for *E. coli* as well. However, no data are available to indicate the frequencies of plasmid transfer *in vivo* by either transduction or transformation.

These observations indicate the low probabilities for possible dissemination of such plasmid vectors by accidental ingestion, which would probably involve only a few hundred or thousand bacteria provided that at least the standard practices (Section II-A above) are followed, particularly the avoidance of mouth pipetting. The possibility of colonization and hence of transfer are increased, however, if the normal flora in the bowel is disrupted by, for example, antibiotic therapy (35). For this reason, persons receiving such therapy must not work with DNA recombinants formed with any *E. coli* K-12 host-vector system during the therapy period and for seven days thereafter; similarly, persons who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work, as should those who require large doses of antacids.

The observations on the fate of *E. coli* K-12 in the human alimentary tract are also relevant to the containment of recombinant DNA formed with bacteriophage λ variants. Bacteriophage can escape from the laboratory either as mature infectious phage particles or in bacterial host cells in which the phage genome is carried as a plasmid or prophage. The fate of *E. coli* K-12 host cells carrying the phage genome as a plasmid or prophage is similar to that for plasmid-containing host cells as discussed above. The survival of the λ phage genome when released as infectious particles depends on their stability in nature, their infectivity and on the probability of subsequent encounters with naturally occurring λ -sensitive *E. coli* strains. Although the probability of survival of λ and its infection of resident intestinal *E. coli* in animals and humans has not been measured, it is estimated to be small given the high sensitivity of λ to the low pH of the stomach, the insusceptibility to λ infection of smooth *E. coli* cells (the type that normally resides in the gut), the infrequency of naturally occurring λ -sensitive *E. coli* (36) and the failure to detect infective λ particles in human feces after ingestion of up to 10^{11} λ particles (37). Moreover, λ particles are very sensitive to desiccation.

Establishment of λ as a stable lysogen is a frequent event (10^0 to 10^{-1}) for the *att⁺ int⁺ cI⁺* phage so that this mode of escape would be the preponderant laboratory hazard; however, most EK1 λ vectors currently in use lack the *att* and *int* functions (27-29) thus reducing the probability of lysogenization to about 10^{-6} to 10^{-8} (38-40). The frequency for the conversion of λ to a plasmid state for persistence and replication is also only about 10^{-6} (41). Moreover, the routine treatment of phage lysates with chloroform (42) should eliminate all surviving bacteria including lysogens and λ plasmid carriers. Lysogenization could also occur

when an infectious λ containing cloned DNA infects a λ -sensitive cell in nature, and recombines with a resident lambdoid prophage. Although λ -sensitive *E. coli* strains seem to be rare, a significant fraction do carry lambdoid prophages (43-44) and thus this route of escape should be considered.

While not exact, the estimates for containment afforded by using these host-vectors are at least as accurate as those for physical containment, and are sufficient to indicate that currently employed plasmid and λ vector systems provide a moderate level of biological containment. Other nonconjugative plasmids and bacteriophages that, in association with *E. coli* K-12 can be estimated to provide the same approximate level of moderate containment are included in the EK1 class.

EK2 host-vectors. These are host-vector systems that have been genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. The genetic modifications of the *E. coli* K-12 host and/or the plasmid or phage vector should not permit survival of a genetic marker carried on the vector, preferably a marker within an inserted DNA fragment, in other than specially designed and carefully regulated laboratory environments at a frequency greater than 10^{-6} . This measure of biological containment has been selected because it is a measurable entity. Indeed, by testing the contributions of preexisting and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible to estimate with considerable precision, that the specially designed host-vector system can provide a margin of biological containment in excess of that required. For the time being, no host-vector system will be considered to be a bona fide EK2 host-vector system until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^4 host cells should be able to perpetuate the vector and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either by survival of the original host or as a consequence of transmission of the vector and/or a cloned DNA fragment by transformation, transduction or conjugation to a host with properties common to those in the natural environment.

In terms of potential EK2 plasmid-host systems, the following types of genetic modifications should reduce survival of cloned DNA. *The examples given are for illustrative purposes and should not be construed to encompass all possibilities.* The presence of the non-conjugative plasmids ColE1-*trp* and pS101 in an *E. coli* K-12 strain possessing a mutation eliminating host-controlled restriction and modification (*hsdS*) results in about 10^2 -fold reduction in mobilization to restriction-proficient recipients. The combination of the *dapD8*, Δ *bioH-asd*, Δ *gal*-

chl⁺ and *rfb* mutations in *E. coli* K-12 results in no detectable survivors in feces of rats following feeding by stomach tube of 10^{10} cells in milk and similarly leads to complete lysis of cells suspended in broth medium lacking diaminopimelic acid. *E. coli* K-12 strains with Δ *thyA* and *deoC* (*dra*) mutations undergo thymineless death in growth medium lacking thymine and give a 10^3 -fold reduced survival during passage through the rat intestine compared to wild-type *thy*⁺ *E. coli* K-12. (However, the Δ *thyA* mutation alone or in combination with a *deoB* (*drm*) mutation only reduces *in vivo* survival by a factor of 10^2 .) Other host mutations, as yet untested, that might further reduce survival of the plasmid-host system or reduce plasmid transmission are: the combination *polA* (TS) *recA* (TS) Δ *thyA* which might interfere with ColE1 replication and lead to DNA degradation at body temperatures; *Con*⁻ mutations that reduce the ability of conjugative plasmids to enter the plasmid-host complex and thus should reduce mobilization of the cloned DNA to other strains; and mutations that confer resistance to known transducing phages. Mutations can also be introduced into the plasmid to cause it to be dependent on a specific host, to make its replication thermosensitive and/or to endow it with a killer capability such that all cells (other than its host) into which it might be transferred will not survive.

In the construction of EK2 plasmid-host systems it is important to use the most stable mutations available, preferably deletions. Obviously, the presence of all mutations contributing to higher degrees of biological containment must be verified periodically by appropriate tests. In testing the level of biological containment afforded by a proposed EK2 plasmid-host system, it is important to design relevant tests to evaluate the survival of the vector and/or a cloned DNA fragment under conditions that are possible in nature and that are also most advantageous for its perpetuation. For example, one might conduct a triparental mating with a primary donor possessing a derepressed F-type or I-type conjugative plasmid, the safer host with Δ *bioH-asd*, *dapD8*, Δ *gal-chl*⁺, *rfb*, Δ *thyA*, *deoC*, *trp* and *hsdS* mutations and a plasmid vector carrying an easily detectable marker such as for ampicillin resistance or an inserted gene such as *trp*⁺, and a secondary recipient that is Su⁺ *hsdS* *trp* (i.e., permissive for the recombinant plasmid). Such matings would be conducted in a medium lacking diaminopimelic acid and thymine and survival of the Ap^r or *trp*⁺ marker in any of the three strains followed as a function of time. Survival of the vector and/or a cloned marker by transduction could also be evaluated by introducing a known generalized transducing phage into the system. Similar experiments should also be done using a secondary recipient that is restrictive for the plasmid vector as well as with primary donors possessing repressed conjugative plasmids with incompatibility group properties like those commonly found in enteric microorgan-

isms. Since a common route of escape of plasmid-host systems in the laboratory might be by accidental ingestion, it is suggested that the same types of experiments be conducted in suitable animal-model systems. In addition to these tests on survival of the vector and/or a cloned DNA fragment, it would be useful to determine the survival of the host strain under nongrowth conditions such as in water and as a function of drying time after a culture has been spilled on a lab bench.

For EK2 host-vector systems in which the vector is a phage, no more than one in 10^4 phage particles should be able to perpetuate itself and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring itself and/or a cloned DNA fragment to a host (or its resident lambdoid prophage) with properties common to those in the natural environment.

In terms of potential EK2 λ -host systems, the following types of genetic modification should reduce survival of cloned DNA. *The examples given are for illustrative purposes and should not be construed to encompass all possibilities.* The probability of establishing λ lysogeny in the normal laboratory host should be reduced by removal of the phage *att* site, the *Int* function, the repressor gene(s) and adding virulence-enhancing mutations. The frequency of plasmid formation, although normally already less than 10^{-6} , could be further reduced by defects in the *p_{re}-Q* region, including mutations such as *vir-s*, *cro* (TS), *c17*, *ri*⁺, *O* (TS), *P* (TS), and *nin*. Moreover, chloroform treatment used routinely following cell lysis would reduce the number of surviving cells, including possible lysogens or plasmid carriers, by more than 10^4 . The host may also be modified by deletion of the host λ *att* site and inclusion of one or more of the mutations described above for plasmid-host systems to further reduce the chance of formation and survival of any lysogen or plasmid carrier cell.

The survival of escaping phage and the chance of encountering a sensitive host in nature are very low, as discussed for EK1 systems. The infectivity of the phage particles could be further reduced by introducing mutations (e.g., suppressed ambers) which would make the phage particles extremely unstable except under special laboratory conditions (e.g., high concentrations of salts or putrescine). Another means would be to make the phage itself a two-component system, by eliminating the tail genes and reproducing the phage as heads packed with DNA; when necessary and under specially controlled conditions, these heads could be made infective by adding tail preparations. An additional safety factor in this regimen is the extreme instability of the heads, unless they are stored in 10mM putrescine, a condition easy to obtain in the laboratory but not in nature. The propagation of the es-

caping phage in nature could further be blocked by adding various conditional mutations which would permit growth only under special laboratory conditions or in a special permissive laboratory host with suppressor or *gro*-type (*mop*, *dnaB*, *rpoB*) mutations. An additional safety feature would be the use of an *r^m-* (*hdsS*) laboratory host, which produces phage with unmodified DNA which should be restricted in *r^m-* bacteria that are probably prevalent in nature. The likelihood of recombination between the λ vector and lambdoid prophages which are present in some *E. coli* strains might be reduced by elimination of the Red function and the presence of the recombination-reducing Gam function together with mutations contributing to the high lethality of the λ phage. However, these second-order precautions might not be relevant if the stability and infectivity of the escaping λ particles are reduced by special mutations or by propagating the highly unstable heads.

Despite multiple mutations in the phage vectors and laboratory hosts, the yield of phage particles under suitable laboratory conditions should be high (10^{10} – 10^{11} particles/ml). This permits phage propagation in relatively small volumes and constitutes an additional safety feature.

The phenotypes and genetic stabilities of the mutations and chromosome alterations included in these λ -host systems indicate that containment well in excess of the required 10^{-8} or lower survival frequency for the λ vector with or without a cloned DNA fragment should be attained. Obviously the presence of all mutations contributing to this high degree of biological containment must be verified periodically by appropriate tests. Laboratory tests should be performed with the bacterial host to measure all possible routes of escape such as the frequency of lysogen formation, the frequency of plasmid formation and the survival of the lysogen or carrier bacterium. Similarly, the potential for perpetuation of a cloned DNA fragment carried by infectious phage particles can be tested by challenging typical wild-type *E. coli* strains or a λ -sensitive nonpermissive laboratory K-12 strain, especially one lysogenic for a lambdoid phage.

In view of the fact that accurate assessment of the probabilities for escape of infections λ -grown on *r^m-* *Su⁺* hosts is dependent upon the frequencies of *r^m-*, *Su⁺*, and λ -sensitive strains in nature, investigators need to screen *E. coli* strains for these properties. These data will also be useful in predicting frequencies of successful escape of plasmid cloning vectors harbored in *r^m-* *Su⁺* strains.

When any investigator has obtained data on the level of containment provided by a proposed EK2 system, these should be reported as rapidly as possible to permit general awareness and evaluation of the safety features of the new system. Investigators are also encouraged to make such new safer cloning systems generally available to other scientists. NIH will take appropriate steps to aid

in the distribution of these safer vectors and hosts.

EK3 host-vectors. These are EK2 systems for which the specified containment shown by laboratory tests has been independently confirmed by appropriate tests in animals, including humans or primates, and in other relevant environments in order to provide additional data to validate the levels of containment afforded by the EK2 host-vector systems. Evaluation of the effects of individual or combinations of mutations contributing to the biological containment should be performed as a means to confirm the degree of safety provided and to further advance the technology of developing even safer vectors and hosts. For the time being, no host-vector system will be considered to be a bona fide EK3 host-vector system, until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

2. Classification of experiments using the *E. coli* K-12 containment systems. In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimums. Higher levels of biological containment (EK3 > EK2 > EK1) are to be used if they are available and are equally appropriate for the purposes of the experiment.

(a) Shotgun Experiments. These experiments involve the production of recombinant DNAs between the vector and the total DNA or (preferably) any partially purified fraction thereof from the specified cellular source.

(i) Eukaryotic DNA recombinants—Primates. P3 physical containment + an EK3 host-vector, or P4 physical containment + an EK2 host-vector, except for DNA from uncontaminated embryonic tissue or primary tissue cultures therefrom, and germ-line cells for which P3 physical containment + an EK2 host-vector can be used. The basis for the lower estimated hazard in the case of DNA from the latter tissues (if freed of adult tissue) is their relative freedom from horizontally acquired adventitious viruses.

Other mammals. P3 physical containment + an EK2 host-vector.

Birds. P3 physical containment + an EK2 host-vector.

Cold-blooded vertebrates. P2 physical containment + an EK2 host-vector except for embryonic or germ-line DNA which require P2 physical containment + an EK1 host-vector. If the eukaryote is known to produce a potent toxin, the containment shall be increased to P3 + EK2.

Other cold-blooded animals and lower eukaryotes. This large class of eukaryotes is divided into the following two groups:

(1) Species that are known to produce a potent toxin or are known pathogens (i.e., an agent listed in Class 2 of ref. 5 or a plant pathogen) or are known to carry such pathogenic agents must use P3 physical containment + an EK2 host-vector. Any species that has a demonstrated capacity for carrying particular

pathogenic agents is included in this group unless it has been shown that those organisms used as the source of DNA do not contain these agents; in this case they may be placed in the second group.

(2) The remainder of the species in this class can use P2 + EK1. However, any insect in this group should have been grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA.

Plants. P2 physical containment + an EK1 host-vector. If the plant carries a known pathogenic agent or makes a product known to be dangerous to any species, the containment must be raised to P3 physical containment + an EK2 host-vector.

(ii) Prokaryotic DNA recombinants—Prokaryotes that exchange genetic information with *E. coli*. The level of physical containment is directly determined by the rule of the most dangerous component (see introduction to Section III). Thus P1 conditions can be used for DNAs from those bacteria in Class 1 of ref. 5 ("Agents of no or minimal hazard ***") which naturally exchange genes with *E. coli*; and P2 conditions should be used for such bacteria if they fall in Class 2 of ref. 5 ("Agents of ordinary potential hazard * * *"), or are plant pathogens or symbionts. EK1 host-vectors can be used for all experiments requiring only P1 physical containment; in fact, experiments in this category can be performed with *E. coli* K-12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. Experiments with DNA from species requiring P2 physical containment which are of low pathogenicity (for example, enteropathogenic *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*) can use EK1 host-vectors, but those of moderate pathogenicity (for example, *Salmonella typhi*, *Shigella dysenteriae* type I, and *Vibrio cholerae*) must use EK2 host-vectors. A specific example of an experiment with a plant pathogen requiring P2 physical containment + an EK2 host-vector would be cloning the tumor gene of *Agrobacterium tumefaciens*.

Prokaryotes that do not exchange genetic information with *E. coli*. The minimum containment conditions for this class consist of P2 physical containment + an EK2 host-vector or P3 physical containment + an EK1 host-vector, and apply when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal. Experiments with DNAs from pathogenic species (Class 2 ref. 5 plus plant pathogens) must use P3 + EK2.

(iii) Characterized clones of DNA recombinants derived from shotgun experiments. When a cloned DNA recombinant has been rigorously characterized "and there is sufficient evidence that it is free of harmful genes," then experiments involving this recombinant DNA can be carried out under P1 + EK1 conditions if the inserted DNA is from a

See footnotes at end of article.

species that exchanges genes with *E. coli*, and under P2 + EK1 conditions if not.

(b) *Purified cellular DNAs other than plasmids, bacteriophages, and other viruses.* The formation of DNA recombinants from cellular DNAs that have been enriched⁵ by physical and chemical techniques (i.e., not by cloning) and which are free of harmful genes can be carried out under lower containment conditions than used for the corresponding shotgun experiment. In general, the containment can be decreased one step in physical containment (P4→P3→P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (EK3→EK2→EK1) while maintaining the specified physical containment—provided that the new condition is not less than that specified above for characterized clones from shotgun experiments (Section (a)—iii).

(c) *Plasmids, bacteriophages, and other viruses.* Recombinants formed between EK-type vectors and other plasmid or virus DNAs have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombinants in *E. coli* K-12 hosts. They do not apply to other hosts where they may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

(i) *Animal viruses.* P4+EK2 or P3EK3 shall be used to isolate DNA recombinants that include all or part of the genome of an animal virus. This recommendation applies not only to experiments of the "shotgun" type but also to those involving partially characterized subgenomic segments of viral DNAs (for example, the genome of defective viruses, DNA fragments isolated after treatment of viral genomes with restriction enzymes, etc.). When cloned recombinants have been shown by suitable biochemical and biological tests to be free of harmful regions, they can be handled in P3+EK2 conditions. In the case of DNA viruses, harmless regions include the late region of the genome; in the case of DNA copies of RNA viruses, they might include the genes coding for capsid proteins or envelope proteins.

(ii) *Plant viruses.* P3+EK1 or P2+EK2 conditions shall be used to form DNA recombinants that include all or part of the genome of a plant virus.

(iii) *Eukaryotic organelle DNAs.* The containment conditions given below apply only when the organelle DNA has been purified⁶ from isolated organelles. Mitochondrial DNA from primates: P3+EK1 or P2+EK2. Mitochondrial or chloroplast DNA from other eukaryotes: P2+EK1. Otherwise, the conditions given under shotgun experiments apply.

(iv) *Prokaryotic plasmid and phage DNAs—Plasmids and phage from hosts that exchange genetic information with *E. coli*.* Experiments with DNA recombinants formed from plasmids or phage genomes that have not been character-

ized with regard to presence of harmful genes or are known to contribute significantly to the pathogenicity of their normal hosts must use the containment conditions specified for shotgun experiments with DNAs from the respective host. If the DNA recombinants are formed from plasmids or phage that are known not to contain harmful genes, or from purified⁶ and characterized plasmid or phage DNA segments known not to contain harmful genes, the experiments can be performed with P1 physical containment + an EK1 host-vector.

*Plasmids and phage from hosts that do not exchange genetic information with *E. coli*.* The rules for shotgun experiments with DNA from the host apply to their plasmids or phages. The minimum containment conditions for this category (P2+EK2, or P3+EK1) can be used for plasmid and phage, or for purified⁶ and characterized segments of plasmid and phage DNAs, when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal.

NOTE.—Where applicable, cDNAs (i.e., complementary DNAs) synthesized *in vitro* from cellular or viral RNAs are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under (a), shotgun experiments; cDNAs formed from purified and characterized RNAs are included under (b); cDNAs formed from viral RNAs are included under (c); etc.

3. *Experiments with other prokaryotic host-vectors.* Other prokaryotic host-vector systems are at the speculative, planning, or developmental stage, and consequently do not warrant detailed treatment here at this time. However, the containment criteria for different types of DNA recombinants formed with *E. coli* K-12 host-vectors can, with the aid of some general principles given here, serve as a guide for containment conditions with other host-vectors when appropriate adjustment is made for their different habitats and characteristics. The newly developed host-vector systems should offer some distinct advantage over the *E. coli* K-12 host-vectors—for instance, thermophilic organisms or other host-vectors whose major habitats do not include humans and/or economically important animals and plants. In general, the strain of any prokaryotic species used as the host is to conform to the definition of Class 1 etiologic agents given in ref. 5 (i.e., "Agents of no or minimal hazard. * * *"), and the plasmid or phage vector should not make the host more hazardous. Appendix A gives a detailed discussion of the *B. subtilis* system, the most promising alternative to date.

At the initial stage, the host-vector must exhibit at least a moderate level of biological containment comparable to EK1 systems, and should be capable of modification to obtain high levels of containment comparable to EK2 and EK3. The type of confirmation test(s) required to move a host-vector from an EK2-type classification to an EK3-type will clearly

depend upon the preponderant habitat of the host-vector. For example, if the unmodified host-vector propagates mostly in, on, or around higher plants, but not appreciably in warm-blooded animals, modification should be designed to reduce the probability that the host-vector can escape to and propagate in, on, or around such plants, or transmit recombinant DNA to other bacterial hosts that are able to occupy these ecological niches, and it is these lower probabilities which must be confirmed. The following principles are to be followed in using the containment criteria given for experiments with *E. coli* K-12 host-vectors as a guide for other prokaryotic systems. Experiments with DNA from prokaryotes (and their plasmids or viruses) are classified according to whether the prokaryote in question exchanges genetic information with the host-vector or not, and the containment conditions given for these two classes with *E. coli* K-12 host-vectors applied. Experiments with recombinants between plasmid or phage vectors and DNA that extends the range of resistance of the recipient species to therapeutically useful drugs must use P3 physical containment + a host-vector comparable to EK1 or P2 physical containment + a host-vector comparable to EK2. Transfer of recombinant DNA to plant pathogens can be made safer by using nonreverting, doubly auxotrophic, non-pathogenic variants. Experiments using a plant pathogen that affects an element of the local flora will require more stringent containment than if carried out in areas where the host plant is not common.

Experiments with DNAs from eukaryotes (and their plasmids or viruses) can also follow the criteria for the corresponding experiments with *E. coli* K-12 vectors if the major habitats of the given host-vector overlap those of *E. coli*. If the host-vector has a major habitat that does not overlap those of *E. coli* (e.g., root nodules in plants), then the containment conditions for some eukaryotic recombinant DNAs need to be increased (for instance, higher plants and their viruses in the preceding example), while others can be reduced.

4. *Experiments with eukaryotic host-vectors—(a) Animal host-vector systems.* Because host cell lines generally have little if any capacity for propagation outside the laboratory, the primary focus for containment is the vector, although cells should also be derived from cultures expected to be of minimal hazard. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by humans; thus vectors should be chosen that have little or no ability to replicate in human cells. To be used as a vector in a eukaryotic host, a DNA molecule needs to display all of the following properties:

(1) It shall not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture.

(2) Its functional anatomy should be known—that is, there should be a clear

⁵ See footnotes at end of article.

idea of the location within the molecule of:

(a) The sites at which DNA synthesis originates and terminates,

(b) The sites that are cleaved by restriction endonucleases,

(c) The template regions for the major gene products.

(3) It should be well studied genetically. It is desirable that mutants be available in adequate number and variety, and that quantitative studies of recombination have been performed.

(4) The recombinant must be defective, that is, its propagation as a virus is dependent upon the presence of a complementing helper genome. This helper should either (a) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector to that particular cell line) or (b) consist of a defective genome or an appropriate conditional lethal mutant virus (in which case the experiments would be done under non-permissive conditions), making vector and helper dependent upon each other for propagation. However, if none of these is available, the use of a non-defective genome as helper would be acceptable.

Currently only two viral DNAs can be considered as meeting these requirements: these are the genomes of polyoma virus and SV40.

Of these, polyoma virus is highly to be preferred. SV40 is known to propagate in human cells, both *in vivo* and *in vitro*, and to infect laboratory personnel, as evidenced by the frequency of their conversion to producing SV40 antibodies. Also, SV40 and related viruses have been found in association with certain human neurological and malignant diseases. SV40 shares many properties, and gives complementation, with the common human papova viruses. By contrast, there is no evidence that polyoma infects humans, nor does it replicate to any significant extent in human cells *in vitro*. However, this system still needs to be studied more extensively. Appendix B gives further details and documentation.

Taking account of all these factors:

(1) *Polyoma Virus.* (a) Recombinant DNA molecules consisting of defective polyoma virus genomes plus DNA sequences of any nonpathogenic organism, including Class 1 viruses (5), can be propagated in or used to transform cultured cells. P3 conditions are required. Appropriate helper virus can be used if needed. Whenever there is a choice, it is urged that mouse cells, derived preferably from embryos, be used as the source of eukaryotic DNA. Polyoma virus is a mouse virus and recombinant DNA molecules containing both viral and cellular sequences are already known to be present in virus stocks grown at a high multiplicity. Thus, recombinants formed *in vitro* between polyoma virus DNA and mouse DNA are presumably not novel from an evolutionary point of view.

(b) Such experiments are to be done under P4 conditions if the recombinant DNA contains segments of the genomes of Class 2 animal viruses (5). Once it

has been shown by suitable biochemical and biological tests that the cloned recombinant contains only harmless regions of the viral genome (see Section IIIB-2-c-i) and that the host range of the polyoma virus vector has not been altered, experiments can be continued under P3 conditions.

(2) *SV40 Virus.*

(a) Defective SV40 genomes, with appropriate helper, can be used as a vector for recombinant DNA molecules containing sequences of any non-pathogenic organism or Class I virus (5), (i.e., a shotgun type experiment). P4 conditions are required. Established lines of cultured cells should be used.

(b) Such experiments are to be carried out in P3 (or P4) conditions if the non-SV40 DNA segment is (a) a purified segment of prokaryotic DNA lacking toxigenic genes, or (b) a segment of eukaryotic DNA whose function has been established, which does not code for a toxic product, and which has been previously cloned in a prokaryotic host-vector system. It shall be confirmed that the defective virus-helper virus system does not replicate significantly more efficiently in human cells in tissue culture than does SV40, following infection at a multiplicity of infection of one or more helper SV40 viruses per cell.

(c) A recombinant DNA molecule consisting of defective SV40 DNA lacking substantial segments of the late region, plus DNA from non-pathogenic organisms or Class I viruses (5), can be propagated as an autonomous cellular element in established lines of cells under P3 conditions provided that there is no exogenous or endogenous helper, and that it is demonstrated that no infectious virus particles are being produced. Until this has been demonstrated, the appropriate containment conditions specified in 2. a. and 2. b. shall be used.

(d) Recombinant DNA molecules consisting of defective SV40 DNA and sequences from non-pathogenic prokaryotic or eukaryotic organisms or Class I viruses (5) can be used to transform established lines of non-permissive cells under P3 conditions. It must be demonstrated that no infectious virus particles are being produced; rescue of SV40 from such transformed cells by co-cultivation or transfection techniques must be carried out in P4 conditions.

(3) Efforts are to be made to ensure that all cell lines are free of virus particles and mycoplasma.

Since SV40 and polyoma are limited in their scope to act as vectors, chiefly because the amount of foreign DNA that the normal virions can carry probably cannot exceed 2×10^6 daltons, the development of systems in which recombinants can be cloned and propagated purely in the form of DNA, rather than in the coats of infectious agents is necessary. Plasmid forms of viral genomes or organelle DNA need to be explored as possible cloning vehicles in eukaryotic cells.

(b) *Plant host-vector systems.* For cells in tissue cultures, seedlings, or plant parts (e.g. tubers, stems, fruits, and de-

tached leaves) or whole mature plants of small species (e.g., *Arabidopsis*) the P1-P4 containment conditions that we have specified previously are relevant concepts. However, work with most plants poses additional problems. The greenhouse facilities accompanying P2 laboratory physical containment conditions can be provided by: (i) Insect-proof greenhouses, (ii) appropriate sterilization of contaminated plants, pots, soil, and runoff water, and (iii) adoption of the other standard practices for microbiological work. P3 physical containment can be sufficiently approximated by confining the operations with whole plants to growth chambers like those used for work with radioactive isotopes, provided that (i) such chambers are modified to produce a negative pressure environment with the exhaust air appropriately filtered, (ii) that other operations with infectious materials are carried out under the specified P3 conditions, and (iii) to guard against inadvertent insect transmission of recombinant DNA, growth chambers are to be routinely fumigated and only used in insect proof rooms. The P2 and P3 conditions specified earlier are therefore extended to include these cases for work on higher plants.

The host cells for experiments on recombinants DNAs may be cells in culture, in seedling or plant parts. Whole plants or plant parts that cannot be adequately contained shall not be used as hosts for shotgun experiments at this time, and attempts to infect whole plants with recombinant DNA shall not be initiated until the effects on host cells in culture, seedlings or plant parts have been thoroughly studied.

Organelle or plasmid DNAs or DNAs of viruses of restricted host range may be used as vectors. In general, similar criteria for selecting host-vectors to those given in the preceding section on animal systems are to apply to plant systems.

DNA recombinants formed between the initial moderately contained vectors and DNA form cells of species in which the vector DNA can replicate, require P2 physical containment. However, if the source of the DNA is itself pathogenic or known to carry pathogenic agents, or to produce products dangerous to plants, or if the vector is an unmodified virus of unrestricted host range, the experiments shall be carried out under P3 conditions.

Experiments on recombinant DNAs formed between the above vectors and DNAs from other species can also be carried out under P2 if that DNA has been purified* and determined not to contain harmful genes. Otherwise, the experiments shall be carried out under P3 conditions if the source of the inserted DNA is not itself a pathogen, or known to carry such pathogenic agents, or to produce harmful products—and under P4 conditions if these conditions are not met.

The development and use of host-vector systems that exhibit a high level of biological containment permit a decrease or one step in the physical con-

*See footnotes at end of article.

tainment specified above (P4→P3→P2→P1).

(c) *Fungal or similar lower eukaryotic host-vector systems.* The containment criteria for experiments on recombinant DNAs using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, in that the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. We therefore consider that the containment guidelines given for experiments with *E. coli* K-12 and other prokaryotic host-vectors (Sections IIIB-1 and -2, respectively) provide adequate direction for experiments with these lower eukaryotic host-vectors. This is particularly true at this time since the development of these host-vectors is presently in the speculative stage.

IV. ROLES AND RESPONSIBILITIES

Safety in research involving recombinant DNA molecules depends upon how the research team applies these guidelines. Motivation and critical judgment are necessary, in addition to specific safety knowledge, to ensure protection of personnel, the public, and the environment.

The guidelines given here are to help the principal investigator determine the nature of the safeguards that should be implemented. These guidelines will be incomplete in some respects because all conceivable experiments with recombinant DNAs cannot now be anticipated. Therefore, they cannot substitute for the investigator's own knowledgeable and discriminating evaluation. Whenever this evaluation calls for an increase in containment over that indicated in the guidelines, the investigator has a responsibility to institute such an increase. In contrast, the containment conditions called for in the guidelines should not be decreased without review and approval at the institutional and NIH levels.

The following roles and responsibilities define an administrative framework in which safety is an essential and integrated function of research involving recombinant DNA molecules.

A. Principal Investigator. The principal investigator has the primary responsibility for: (i) Determining the real and potential biohazards of the proposed research, (ii) determining the appropriate level of biological and physical containment, (iii) selecting the microbiological practices and laboratory techniques for handling recombinant DNA materials, (iv) preparing procedures for dealing with accidental spills and overt personnel contamination, (v) determining the applicability of various precautionary medical practices, serological monitoring, and immunization, when available, (vi) securing approval of the proposed research prior to initiation of work, (vii) submitting information on purported EK2 and EK3 systems to the NIH Recombinant DNA Molecule Program Advisory Committee and making the strains available to others, (viii) reporting to the institutional biohazards committee and the NIH Office of Recombinant DNA

Activities new information bearing on the guidelines, such as technical information relating to hazards and new safety procedures or innovations, (ix) applying for approval from the NIH Recombinant DNA Molecule Program Advisory Committee for large scale experiments with recombinant DNAs known to make harmful products (i.e., more than 10 liters of culture), and (x) applying to NIH for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes.

Before work is begun, the principal investigator is responsible for: (1) Making available to program and support staff copies of those portions of the approved grant application that describe the biohazards and the precautions to be taken, (ii) advising the program and support staff of the nature and assessment of the real and potential biohazards, (iii) instructing and training this staff in the practices and techniques required to ensure safety, and in the procedures for dealing with accidentally created biohazards, and (iv) informing the staff of the reasons and provisions for any advised or requested precautionary medical practices, vaccinations, or serum collection.

During the conduct of the research, the principal investigator is responsible for: (i) Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed, (ii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any serious or extended illness of a worker or any accident that results in (a) inoculation of recombinant DNA materials through cutaneous penetration, (b) ingestion of recombinant DNA materials, (c) probable inhalation of recombinant DNA materials following gross aerosolization, or (d) any incident causing serious exposure to personnel or danger of environmental contamination, (iii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any problems pertaining to operation and implementation of biological and physical containment safety practices and procedures, or equipment or facility failure, (iv) correcting work errors and conditions that may result in the release of recombinant DNA materials, and (v) ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.).

B. Institution. Since in almost all cases, NIH grants are made to institutions rather than to individuals, all the responsibilities of the principal investigator listed above are the responsibilities of the institution under the grant, fulfilled on its behalf by the principal investigator. In addition, the institution is responsible for establishing an institutional biohazards committee⁷ to: (i) Advise the

institution on policies, (ii) create and maintain a central reference file and library of catalogs, books, articles, newsletters, and other communications as a source of advice and reference regarding, for example, the availability and quality of the safety equipment, the availability and level of biological containment for various host-vector systems, suitable training of personnel and data on the potential biohazards associated with certain recombinant DNAs, (iii) develop a safety and operations manual for any P4 facility maintained by the institution and used in support of recombinant DNA research, (iv) certify to the NIH on applications for research support and annually thereafter, that facilities, procedures, and practices and the training and expertise of the personnel involved have been reviewed and approved by the institutional biohazards committee.

The biohazards committee must be sufficiently qualified through the experience and expertise of its membership and the diversity of its membership to ensure respect for its advice and counsel. Its membership should include individuals from the institution or consultants, selected so as to provide a diversity of disciplines relevant to recombinant DNA technology, biological safety, and engineering. In addition to possessing the professional competence necessary to assess and review specific activities and facilities, the committee should possess or have available to it, the competence to determine the acceptability of its findings in terms of applicable laws, regulations, standards of practices, community attitudes, and health and environmental considerations. Minutes of the meetings should be kept and made available for public inspection. The institution is responsible for reporting names of and relevant background information on the members of its biohazards committee to the NIH.

C. NIH Initial Review Groups (Study Sections). The NIH Study Sections, in addition to reviewing the scientific merit of each grant application involving recombinant DNA molecules, are responsible for: (i) Making an independent evaluation of the real and potential biohazards of the proposed research on the basis of these guidelines, (ii) determining whether the proposed physical containment safeguards certified by the institutional biohazards committee are appropriate for control of these biohazards, (iii) determining whether the proposed biological containment safeguards are appropriate, (iv) referring to the NIH Recombinant DNA Molecule Program Advisory Committee or the NIH Office of Recombinant DNA Activities those problems pertaining to assessment of biohazards or safeguard determination that cannot be resolved by the Study Sections.

The membership of the Study Sections will be selected in the usual manner. Biological safety expertise, however, will be available to the Study Sections for consultation and guidance.

D. NIH Recombinant DNA Molecule Program Advisory Committee. The Recombinant DNA Molecule Program Advisory Committee advises the Secretary, Department of Health, Education, and

⁷ See footnotes at end of article.

Welfare, the Assistant Secretary for Health, Department of Health, Education, and Welfare, and the Director, National Institutes of Health, on a program for the evaluation of potential biological and ecological hazards of recombinant DNAs (molecules resulting from different segments of DNA that have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of their host's genome), on the development of procedures which are designed to prevent the spread of such molecules within human and other populations, and on guidelines to be followed by investigators working with potentially hazardous recombinants.

The NIH Recombinant DNA Molecule Program Advisory Committee has responsibility for: (i) Revising and updating guidelines to be followed by investigators working with DNA recombinants, (ii) for the time being, receiving information on purported EK2 and EK3 systems and evaluating and certifying that host-vector systems meet EK2 or EK3 criteria, (iii) resolving questions concerning potential biohazard and adequacy of containment capability if NIH staff or NIH Initial Review Group so request, and (iv) reviewing and approving large scale experiments with recombinant DNAs known to make harmful products (e.g., more than 10 liters of culture).

E. NIH Staff. NIH Staff has responsibility for: (i) Assuring that no NIH grants or contracts are awarded for DNA recombinant research unless they (a) conform to these guidelines, (b) have been properly reviewed and recommended for approval, and (c) include a properly executed Memorandum of Understanding and Agreement, (ii) reviewing and responding to questions or problems or reports submitted by institutional biohazards committees or principal investigators, and disseminating findings, as appropriate, (iii) receiving and reviewing applications for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, (iv) referring items covered under (ii) and (iii) above to the NIH Recombinant DNA Molecule Program Advisory Committee, as deemed necessary, and (v) performing site inspections of all P4 physical containment facilities, engaged in DNA recombinant research, and of other facilities as deemed necessary.

V. FOOTNOTES

¹ Biological Safety Cabinets referred to in this section are classified as *Class I*, *Class II* or *Class III* cabinets. A *Class I* cabinet is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency or high efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is used in three operational modes; (1) with an 8 inch high full width open front, (2) with an installed front closure panel (having four eight inch diameter

openings) without gloves, and (3) with an installed front closure panel equipped with arm length rubber gloves. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all Biohazard Safety Cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air filtered through HEPA filters. Exhaust air is filtered through HEPA filters or incinerated before being discharged to the outside environment.

² Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection and/or conjugation with transfer of phage, plasmid and/or chromosomal genetic information.

³ The bacteria which constitute Class 2 of ref. 5 ("Agents of ordinary potential hazard * * *") represent a broad spectrum of etiologic agents which possess different levels of virulence and degrees of communicability. We think it appropriate for our specific purpose to further subdivide the agents of Class 2 into those which we believe to be of relatively low pathogenicity and those which are moderately pathogenic. The several specific examples given may suffice to illustrate the principle.

⁴ The terms "characterized" and "free of harmful genes" are unavoidably vague. But, in this instance, before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the National Institutes of Health. Such approval would be contingent upon data concerning: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector.

⁵ A DNA preparation is defined as enriched if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation. The reason for lowering the containment level when this degree of enrichment has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10⁶-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

⁶ The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

⁷ In special circumstances, in consultation with the NIH Office of Recombinant DNA Activities, an area biohazards committee may be formed, composed of members from the institution and/or other organizations be-

yond its own staff, as an alternative when additional expertise outside the institution is needed for the indicated reviews.

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VII. MEMBERS OF THE RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

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APPENDIX A—STATEMENT ON THE USE OF "Bacillus subtilis" IN RECOMBINANT MOLECULE TECHNOLOGY

Unquestionably, *Escherichia coli* is the most well characterized unicellular organism. Years of basic research have enabled investigators to develop a well characterized genetic map, to obtain detailed knowledge of virulent and temperate bacteriophages, and to explore the physiology, genetics, and regulation of plasmids. More recently, the development of DNA-mediated transformation has permitted exogenous fragments or molecules of DNA to be incorporated into the genome or to reside as self-replicating units. The discovery of transformation of *Bacillus subtilis* by Spizizen (1) stimulated the development of an alternative model system. The purpose of this report is to summarize the current status of this genetic system and to describe the actual and potential vectors and vehicles available for recombinant molecule technology.

A. CURRENT KNOWLEDGE OF THE CHROMOSOMAL ARCHITECTURE AND MECHANISMS OF GENETIC EXCHANGE IN B. "SUBTILIS"

Two mechanisms of genetic exchange have been utilized to establish the linkage map of *B. subtilis*, DNA-mediated transformation (capable of transferring approximately 1% of the genome) and transduction with bacteriophage PBS1 (capable of transferring 5-8% of the chromosome). Recent detailed genetic studies with PBS1 by Lepesant-Kejzlorová et al. (2) have resulted in the development of a circular genetic map for this organism. The current edition of the map (3) contains 196 loci. Biophysical analyses have established that the chromosome is circular (4) and replicates bidirectionally (5).

Transformation with purified fragments of DNA is a highly efficient process in *B. subtilis* with frequencies of 1 to 4% usually attained for any auxotrophic or antibiotic resistance markers. Frequencies of approximately 10% transformation can be achieved with DNA prepared from gently lysed L-forms or protoplasts (6). These large fragments of DNA are readily incorporated by the recipient cell. Generalized transduction occurs with bacteriophages SP10 (7), PBS1 (8), and SPPI (9), while a low frequency of specialized transduction has been reported with bacteriophage ϕ 105 (10).

Although transformation is most efficient in homologous crosses (*B. subtilis* into *B. subtilis*), it has also been possible to exchange DNA among closely related species (11). The most extensively studied members of the *B. subtilis* species include *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, and *B. globigii* (refer to reference 12 for a review and references 13-15 for examples of this heterologous exchange). This exchange occurs even

though there is a surprisingly wide discrepancy between DNA-DNA hybridization among these organisms (16). Even though the frequency of transformation is low in the heterologous cross [e.g., *B. amyloliquefaciens* (donor)/*B. subtilis* (recipient)], the newly acquired DNA from *B. amyloliquefaciens* in the *B. subtilis* background can be readily transferred at high efficiencies to other recipient strains of *B. subtilis* (14). Therefore, the extremely high frequency of transformation permits the recognition and selection of rare events.

B. CURRENT AND POTENTIAL VECTORS FOR RECOMBINANT MOLECULE EXPERIMENTS

Lovett and coworkers have recently described cryptic plasmids in *B. pumilus* (17) and *B. subtilis* (18). Of these organisms, *B. subtilis* ATCC 7003 appears to be the most useful since it carries one to two copies of a plasmid with a molecular weight of 4.6×10^6 . This strain is also closely related to *B. subtilis* 168. Another strain of *B. subtilis* (ATCC 15841) contains 16 copies of a plasmid with a molecular weight of 4.6×10^6 . Currently it is not known whether genetic markers can be readily introduced into these plasmids. To date it has not been possible to readily stabilize plasmids derived from *B. pumilus* in *B. subtilis* even with heavy selective pressure (P. Lovett, personal communication).

Two temperate bacteriophages are under development as vectors in *B. subtilis*, ϕ 3T and SP02. Lysogeny of thymine auxotrophs (strains carrying *thyA* *thyB*) by bacteriophage ϕ 3T results in "conversion" to a *Thy*⁺ phenotype. The attachment site for this bacteriophage and the bacteriophage gene for thymidylate synthetase (*thyP*) map between the bacterial *thyA* and *thyB* loci in the terminal region of the chromosome of *B. subtilis* (19). The viral genome is readily cleaved by the site-specific endonuclease, Bam 1 (20), to produce 5 fragments (one of which carries the *thyP* gene). The *thyP* carrying gene can be integrated into the bacterial genome in the absence of the intact viral genome. Because deletions are available that include the *thyP* region, it is theoretically possible to introduce *thyP* at many sites on the chromosome. The *thyP* gene can be readily purified for insertion into plasmids or utilized as a scaffold to integrate other heterologous DNA into the chromosome of *B. subtilis*. Alternatively, it is possible to purify fragments of the chromosome by gel electrophoresis (21, 22), for insertion into bacteriophage ϕ 3T or SP02. At present, unfortunately, only the former carries a selective marker, i.e., the gene for thymidylate synthetase, *thyP*.

C. DEVELOPMENT OF VEHICLES

B. subtilis is a Gram-positive sporulating rod that usually inhabits soil. Although it can exist on cutaneous surfaces of man (23) and experimental animals, it rarely produces disease. To develop a suitable vehicle it is imperative to have a host that is asporogenic. The most appropriate deletion mutation is deletion 29 (cit D). In addition to a deficiency in sporulation this mutant rapidly lyses when it has reached the end of its growth cycle. Presumably this is due to the failure to inactivate one of the autolytic enzymes (24). Through the introduction of a D-alanine requirement (34 μ g/ml) it is possible to block transport of compounds that are transported by active transport (25, 26). The further introduction of thymine auxotrophy (defects in the *thyA* *thyB* loci) will enable the strain to survive only with a plasmid vector carrying the purified *thyP* gene from bacteriophage ϕ 3T or a defective bacteriophage ϕ 3T carrying the *thyP* gene but attached to the chromosome at an alternative site (due to the presence of deletion

29 in the host). We have recently isolated temperature-sensitive *thyP* mutants. If we can isolate a temperature-dependent lysogen that will grow only at 48°C it should be possible to make an unusual vehicle.

D. SITE-SPECIFIC ENDONUCLEASES

Recently two restriction modification systems have been observed between *B. subtilis* 168 and other bacilli. Trautner *et al.* have isolated an effective system that inhibits infection of the R strain of *B. subtilis* by bacteriophage SP1 propagated on *B. subtilis* 168 (27). The site-specific nuclease recognizes the sequence GGCC. Young, Radnay, and CCGC

Wilson observed a restriction modification system between *B. amyloliquefaciens* and *B. subtilis* 168 (28). The endonuclease from *B. amyloliquefaciens* (20) recognizes the sequence GGATCC (29). More recently, two additional enzymes have been isolated from *B. globigii* (30). The recognition sequence is not known.

E. ADVANTAGES AND LIABILITIES OF THE B. "SUBTILIS" SYSTEM

a. Advantages

1. *B. subtilis* is nonpathogenic. Asporogenic deletion mutants are available to preclude the problem of persistence through sporulation.
2. The circular chromosomal map is well defined. At least 196 loci have been positioned.
3. The organism is commercially important in the fermentation industry.
4. Large numbers of organisms can be disposed of readily with minimal environmental impact.
5. Unlike *E. coli*, it lacks endotoxin in the cell wall. Therefore the cells can be used as a single cell protein source.
6. The frequency of transformation is very high, facilitating the detection of rare events.
7. A unique bacteriophage, ϕ 3T, exists that carries a gene that can be readily purified for "scaffolding" experiments.

b. Disadvantages

1. The knowledge of genetics and physiology of plasmids and viruses is primitive compared with *E. coli*.
2. High-frequency, specialized transduction is not available as a means of gene enrichment.

Based on its promise, it seems appropriate, and not chauvinistic, to urge development of this system.

Prepared by: Dr. Frank Young, University of Rochester.

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APPENDIX B.—POLYOMA AND SV40 VIRUS

Polyoma virus is a virus of mice, and infection of wild mouse populations is a common event, for the virus has often been isolated from a high proportion of healthy adult animals, both wild and laboratory bred, of many colonies (Gross, L., *Proc. Soc. Exp. Biol.* 88, 362-368, 1955; Rowe, W. P., *Bact. Rev.* 25, 18-31, 1961). As far as is known the virus almost never causes a disease in these animals. However, when large quantities of the virus are inoculated into newborn or suckling mice or hamsters, a variety of solid tumors is induced (Gross, L., *Oncogenic Viruses*, Second Edition, Pergamon Press, NY).

Polyoma virus grows lytically in mouse cells in tissue culture. Thus mouse cells in culture are probably transformed only by virus particles that contain certain kinds of defective genomes. Cells of other rodent species, however, can be transformed by polyoma virus particles that contain complete genomes (Folk, W., *J. Virol.* 11, 424-431, 1973). The virus does not replicate to a significant extent in human cells in tissue culture (Eddy, B. E., *Virol. Monogr.* 7, 1-114, 1969; Pollack, R. E., Salas, J., Wang, R., Kusano, T., and Green, H., *J. Cell Physiol.* 77, 117-120, 1971). The resistance of the cells seems to be a consequence of the failure of the virus to absorb or uncoat. However even when naked viral DNA is introduced into the cells only an abortive cycle of replication ensues; early viral proteins are made, there is induction of cellular DNA synthesis, but no expression of late viral proteins is detectable (Gruen, R., Grassmann, M., and Grassmann, A., *Virology*, 58, 290-293, 1974).

There is no evidence that polyoma virus can infect humans (Hartley, J., Huebner, R., Parker, J., and Rowe, W. P., unpublished data). Thus no antibodies to the virus have been detected in people living in buildings that are infested with virus-infected mice, nor in laboratory workers who have been exposed to the virus for a number of years.

At most, a small segment of polyoma virus DNA shows weak homology with a portion of the late region of SV40 DNA (Ferguson, J., and Davis, R. W., *J. Biol. Biol.*, 94, 135-150, 1975). However, there appears to be no genetic interaction between the two viruses and there is no immunological cross-reaction between the gene products of the two viruses.

SV40 causes persistent but apparently harmless infections of the kidneys of virtually all adult rhesus monkeys (Hsiung, G. D., *Bact. Revs.* 32, 185-205, 1968), it causes tumors when injected into newborn hamsters (Girardi, A. J., Sweet, B. H., Slotnick, V. B., and Hillemann, M. R., *Proc. Soc. Exp. Biol. Med.*, 105, 420-427, 1964) and transforms cells of several mammalian species (including human). SV40 is able to infect human since antibodies to the virus are found in a small proportion of the human population (Shah, K. V., Goverdhan, M. K., and Ozer, H. L., *Am. J. Epid.* 93, 291-298, 1970) and serum conversions have been noted in many laboratory personnel who have been exposed to the virus (Horvath, L. B., *Acta Microbiol. Acta Sci. Hung.* 12, 201-206, 1965).

Isolations of SV40 have been reported from humans, twice from patients suffering from the rare demyelinating disease, progressive multifocal leukoencephalopathy (Weiner, L., Herndon, R., Narayan, O., Johnson, R. T.,

Shah, K., Rubinstein, L. G., Preziosi, T. J., and Conley, F. K., *New England J. Med.* 286, 385-390, 1972) and apparently from a tumor of a person with metastatic melanoma (Sorian, F., Shelburne, C. E., and Gokcen, M., *Nature*, 249, 421-424, 1974). In other studies a non-structural antigen characteristic of papovaviruses, T antigen, has been detected in the nuclei of cells cultured from 2 meningiomas, while another SV40-specific antigen, U antigen, has been found in the cells of a third tumor of the same type (Weiss, A. F., Portman, R., Fisher, H., Simon, J., and Zang, K. D., *Proc. Nat. Acad. Sci. USA* 72, 609-613, 1975). Furthermore new papovaviruses have been isolated from the brains of patients with PML (JC virus—Padgett, B. L., Walker, D. L., zur Rhein, G. M., Eckroade, R. I., and Dessel, B. H., *Lancet* 1, 1257-1260, 1971), from the urine of a patient carrying a renal allograft (BK virus—Gardner, S. D., Field, A. M., Coleman, D. V., and Hulme, B., *Lancet* 1, 1253-1257, 1971) and from a reticulum cell sarcoma and the urine of patients with the sex-linked recessive disorder, Wiscott-Aldrich syndrome (Takemoto, K. K., Rabson, A. S., Mullarkey, M. F., Blaese, R. M., Garon, C. F., and Nelson, D. J., *Nat. Cancer Inst.*, 53, 1205-1207, 1974). All of these viruses which are distributed widely throughout human populations share antigenic and biological properties with SV40; the virus particles are identical in size and architecture (Madeley, C. R., In *Virus Morphology*, Churchill-Livingstone, London, 134-135, 1972); the non-structural intracellular T antigen, which appears to be coded by the A gene of SV40 cross reacts extensively with antigens found in cells infected or transformed by BK or JC viruses; both JC or BK viruses induce tumors in newborn hamsters (Walter, D. L., Padgett, B. L., zur Rhein, B. M., Albert, A. E. and Marsh, R. F., *Science* 181, 674-676, 1973; Shah, K. V., Daniel, R. W., and Strandberg, J., *J. Nat. Cancer Inst.* 54, 945-950, 1975); BK virus causes transformation of hamster cells in culture (Major, E. D., and DiMayora, G., *Proc. Nat. Acad. Sci. USA*, 70, 3210-3212, 1973; Portolani, M., Barbanti, A., Brodano, G., and LaPlaca, M. J., *Virol.* 15, 420-422, 1975) and is able to complement the growth of certain temperature-sensitive mutants of SV40 (Mason, D. H. and Takemoto, K. K., submitted for publication).

FURTHER WORK

At present, a potential eukaryotic vector of choice is polyoma virus. And while available information indicates that it fulfills all the necessary criteria, we recommend that the following subjects be further investigated:

1. The molecular mechanism of resistance of human cells to the virus.
2. The extent of homology between polyoma virus DNA and the DNAs of human papovaviruses.
3. The ability of human papovaviruses to complement defective polyoma virus genomes.

Report of a working group consisting of: Dr. Bernard Fields, Harvard University School of Medicine; Dr. Thomas J. Kelly, Jr., Johns Hopkins University School of Medicine; Dr. Andrew Lewis, National Institute of Allergy and Infectious Diseases; Dr. Malcolm Martin, National Institute of Allergy and Infectious Diseases; Dr. Robert Martin, National Institute of Arthritis, Metabolism, and Digestive Diseases; Dr. Elmer Pfefferkorn, Dartmouth Medical School; Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases; Dr. Aaron Shatkin, Roche Institute of Molecular Biology; Dr. Maxine Singer, National Cancer Institute. Rapporteur: Dr. Joe Sambrook, Cold Spring Harbor Laboratory.

APPENDIX C.—SUMMARY OF THE WORKSHOP ON THE DESIGN AND TESTING OF SAFER PROKARYOTIC VEHICLES AND BACTERIAL HOSTS FOR RESEARCH ON RECOMBINANT DNA MOLECULES

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The development of techniques for the cloning of DNA from both prokaryotic and eukaryotic organisms in bacteria has had great impact on research in biology and medicine and promises extraordinary social benefits. The biohazards involved in the use of this technology in many instances are very difficult to assess. For this reason codes of practice are being formulated in the United States and other countries for the conduct of those experiments that present a potential biohazard. One of the requirements for conducting certain cloning experiments is the use of safer vector (bacteriophage or plasmid)—host systems, i.e., vector-bacterium systems that have restricted capacity to survive outside of controlled conditions in the laboratory. Approximately sixty scientists from the United States and several foreign countries participated in a workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules at La Jolla, California, on 1 to 3 December, 1975. The workshop was sponsored by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. The purposes of the meeting were the exchange of recent data on the development of safer prokaryotic host-vector systems, devising methods of testing the level of containment provided by these systems and exploring the various directions that future research should take in the construction of safer bacterial systems for the cloning of foreign DNA.

The first session of the workshop, chaired by W. Szybalski (University of Wisconsin), was devoted to bacteriophage vectors. Szybalski outlined the main safety features of the two-component, phage-bacterial system, in which the host bacteria offer the safety feature of not carrying the cloned DNA, and the phage vectors cannot be propagated in the absence of an appropriate host. There are two primary escape routes for the clones of foreign DNA carried by the phage vector: (1) establishment of a stable prophage or plasmid in the laboratory host used for phage propagation, and subsequent escape of this self replicating lysogen or carrier system, and (2) escape of the phage vector which carries the cloned DNA and its subsequent productive encounter with a suitable host in the natural environment. The general consensus was that to ensure safety, both routes should be blocked by appropriate genetic modifications. For phage λ , route (1) can be blocked by phage mutations that interfere with lysogenization (*att⁻*, *int⁻*, *cI⁻*, *cIII⁻*, *vir⁻*) and plasmid formation (*N⁻*, *nitR⁻*, *vS⁻*, *ri⁻*, *c17⁻*, *Ots*, *crots*), and by mutations on the *Escherichia coli* host that affect these processes (*attB⁻*, *dncA*s) and host survival. Route (2), [which is of low probability since λ phages do not survive well in natural environments (no λ CI phage was recovered after ingestion of 10^8 - 10^{11} particles), are killed by desiccation, and have a low chance to encounter a naturally sensitive host] can be blocked further by the following phage modifications: (a) mutations which result in extreme instability of the infectious phage particles under all conditions other than those specially designed for phage propagation in the laboratory (e.g., high concentrations of putrescine or some other compound), or (b) employing phage vectors in which the tail genes are deleted and which permit propagation of only the DNA-packed heads; only under laboratory conditions could such heads be made transiently infec-

tious by rejoining them with separately prepared tails. The high instability of the phage would minimize the possibility of transfer of the cloned genes into receptive bacteria found in nature. Moreover, the propagation of the phage can be blocked by many conditional mutations, which would be designed to block any secondary route of escape, mainly depending on transfer of the cloned DNA into another phage or bacterial host. It was recommended further that the vector be designed in such a manner as to permit easy insertion and monitoring of the foreign DNA and rapid assay of the safety features and give a high yield of cloned DNA (not less than 10^{11} molecules per ml). There also was general agreement that host-phage systems other than *E. coli* should be considered, especially those restricted to very rare and unusual environments. Also, plasmids derived from phage vectors and which give very high DNA yields while exhibiting safety features, e.g., λ derivots, should be considered as vehicles for cloned DNA.

Szybalski and S. Brenner (Cambridge University) stressed that research on recombinant DNA molecules may lend itself to very simple and inexpensive mechanical containment, e.g., a small sealed glove box, since all the vectors that carry such recombinant molecules possibly can be both created and destroyed in such a box, while development of special methods might permit study of many properties of the recombinant DNA, without ever removing it from the box.

These safety features were reflected in the subsequent presentations. F. Blattner and W. Williams (University of Wisconsin) described four specially constructed λ - ϕ 80 phages which incorporate many of these safety features, and which they named Charon phages, for the mythical boatman of the river Styx. Some of these highly contained phages give yields of over 10^{11} particles/ml. R. Davis, J. Cameron and K. Struhl (Stanford University) found that λ phages that carry foreign DNA never grow as well as the parental vector, which would select against their survival in nature. They also reported that some eukaryotic genes could be expressed in *E. coli*, partially compensating for deficiencies in the histidine pathway or in *polA* or *hly* functions. These investigators surveyed over 1000 strains of *E. coli* isolated in the natural environment and did not find a single strain that could support propagation of the λ vir vector.

V. Bode (Kansas State University) discussed the possibility of growing tail-free λ heads. Such heads, which are packed with DNA, are very fragile, unless stored in 0.01 M putrescine buffer. Head yields close to 10^{11} /ml could easily be attained and, when required, heads could be quantitatively rejoined with separately supplied tails under special laboratory conditions. W. Arber, D. Scandella and J. Elliott (University of Basel) described bacterial host mutants that permit efficient infection only by phages with a full complement of DNA. This permits selecting for vectors that carry long fragments of foreign DNA.

K. Matsubara, T. Mukai and Y. Takagi (University of Osaka and Kyushu University), and G. Hobom and P. Philippson (University of Freiburg and Stanford University) described various defective λ plasmids (λ dv) that could be used as efficient vectors. Matsubara has shown that temperature-sensitive *cro* mutations permit obtaining between 1000 and 3000 cloned molecules per cell and at the same time result in killing of the carrier cells at body temperature. The mutations *Ots* and *Pts* were also evaluated as safety features. Philippson described many new λ dv plasmids created by cutting λ DNA with *HindIII* and *BamI* restriction endonucleases followed by ligation. The final talk

by F. Young, G. Wilson and M. Williams (University of Rochester) summarized the progress on the development of safer *Bacillus subtilis* host mutants and phages, especially ϕ 3, as vectors. New restriction nucleases, Bgl-1 and Bgl-2, were also described.

The morning session on bacteriophage vectors was followed by a session on plasmid vectors that was chaired by D. Hellinski (University of California, San Diego). Hellinski presented the following properties as highly desirable characteristics of a safer plasmid vehicle: (a) non-conjugative; (b) non-mobilizable or poorly mobilizable by a conjugative plasmid; (c) possesses little or no extraneous genetic information; (d) poorly recombines or does not recombine with the chromosome of the host cell; (e) provides no selective advantage to the host cell or the selective property is conditional; and (f) possesses mutations that restrict its maintenance to a specific host, prevent replication at mammalian body temperature and/or provide the plasmid with the capability of killing any cell to which it might be transmitted other than the host cell. V. Hershfield (University of California, San Diego) described the properties of a variety of derivatives of the ColE1 plasmid and the broad-host range, P-type plasmid, RK2. One of the ColE1 derivatives, ColE1-*trp*, constructed in collaboration with C. Yanofsky and N. Franklin (Stanford University) provides the means to use the tryptophan genes of *E. coli* as a selective marker in transformation with recombinant DNA in situations where it is desirable to avoid antibiotic resistance genes. In addition, Hershfield described collaborative work with H. Boyer that resulted in the development of a mini-ColE1 plasmid and derivatives of this plasmid (mini-ColE1-*kan* and mini-ColE1-*trp*) as cloning vehicles. Finally, she described the temperature-sensitivity properties of *trp* and *kan* derivatives of a temperature sensitive replication mutant of ColE1 isolated by J. Collins (Molecular Biology Institute, Stockholm) and hybrid ColE1 plasmids carrying the *EcoRI* generated Cts fragment of bacteriophage λ -*trp*61.

J. Carbon (University of California, Santa Barbara) described a replica plating method that greatly facilitates the detection of *E. coli* clones bearing ColE1 plasmids. The procedure, which utilizes the *F'* plasmid to promote the transfer of a hybrid ColE1 plasmid to a suitable auxotrophic recipient, was successful in identifying clones bearing hybrid plasmids carrying a number of different regions of the *E. coli* chromosome. The contributions of A. J. Clark and collaborators (University of California, Berkeley) were relevant to the problem of the mobilization and subsequent transfer of non-conjugative plasmids carrying foreign DNA of a potentially hazardous nature. Clark described the variations in transmission frequencies between the nonconjugative plasmids pSC101, pML31, pSC138 and a number of pSC101 hybrids containing various *EcoRI* fragments of *F* when the conjugal transfer of these plasmids was promoted by several different conjugative plasmids.

I. C. Gunsalus and collaborators (University of Illinois) and A. Chakrabarty (General Electric Research and Development Center) described the properties of a variety of plasmids isolated from *Pseudomonas putida*. These contributions were followed by a discussion on the merits of developing plasmid-host systems involving *Pseudomonas* strains that naturally exhibit unusual growth requirements. Similar studies with plasmids isolated from *Bacillus megaterium* by B. Carlton (University of Georgia) from *B. subtilis* by P. Lovett (University of Maryland) and other naturally occurring *Bacillus* species by W. Goebel and K. Bernhard (Mi-

crobiology Institute, Wuerzburg) were discussed and their further development as plasmid-host cloning systems was explored. It was clear from these presentations that considerable progress has been made recently in the identification and characterization of a variety of plasmid elements that occur naturally in *Pseudomonas* and *Bacillus* species. Several of the plasmids described show considerable promise as plasmid cloning systems involving a host other than *E. coli*.

A third session on the ecology and epidemiology of vector-host systems was chaired by S. Falkow (University of Washington). This workshop emerged, in part, from expressed fears that microorganisms containing cloned fragments of foreign DNA potentially pose a threat to health or disrupt the normal ecological chain in some manner. Consequently, this session was devoted to a review of currently available information on the ecology and epidemiology of *E. coli* and related bacterial species since it was recognized that *E. coli* K-12 would be the prokaryotic host most commonly employed in the cloning of DNA molecules in the immediate future. F. Orskov (Escherichia Reference Center, Copenhagen) reviewed the state of *E. coli* serotyping and what has been learned about the distribution of *E. coli* types in health and disease. Only certain *E. coli* types are generally recognized as good colonizers of the human gut and such strains come from a handful of the 160 well defined O (lipopolysaccharide) antigen types and invariably possess K (acidic polysaccharide capsule) antigens. Some serotypes apparently have become disseminated worldwide and possibly represent the proliferation of a bacterial clone because of, as yet unknown, selective pressures. In contrast, *E. coli* K-12 has no detectable O or K antigens and is considered to be rough. This may account, at least in part, for its demonstrable poor ability to colonize the human or animal gut. However, R. Freter (University of Michigan) pointed out that we still remain largely ignorant of the factors which control intestinal *E. coli* populations. Freter also noted that while adherence to the mucosal surface of the small intestine is important in the pathogenesis of *E. coli* diarrheal disease, the "normal" long-lasting symbiotic relationship between a mammalian host and bacterium is established in the cecum and colon. It is in these locations that factors come into play to determine whether an *E. coli* strain passing through the intestine will become successfully implanted or whether it will be quickly eliminated in the feces.

The factors controlling implantation include competition for substrates, inhibitors and the physiological state of the organism when it reaches the large bowel. For example, ingested *E. coli* previously grown under usual laboratory conditions fare poorly while cells of the same strain "pre-adapted" in Eh, pH, etc., often colonize well. Freter has developed a continuous flow culture model which may be useful in studying the mechanisms of implantation. Falkow reviewed the pathogenicity of *E. coli*. *E. coli* causes diarrheal disease either by direct invasion of the bowel epithelium or by elaboration of enterotoxin(s). While invasive *E. coli* appear to owe their pathogenicity to a constellation of at least five unlinked chromosomal gene clusters, toxigenic *E. coli* species generally owe their pathogenicity to the possession of two species, Ent and K. The introduction of Ent and K plasmids may be sufficient to convert a normal wild-type *E. coli* into a strain now capable of causing overt clinical disease. However, the introduction of these plasmids into *E. coli* K-12 sublines had no discernible effect on their ability to cause disease, although the K-12 strains could now better colonize calves. Despite the observation that

E. coli K-12 did not appear to offer a significant hazard as a potential enteric pathogen even when it possessed well-defined determinants of pathogenicity it was emphasized by Orskov, Freter and Falkow that *E. coli* K-12 strains carrying recombinant DNA molecules could still act as effective genetic donors *in vivo* and still posed a significant problem requiring control. E. Geldreich (U.S. Environmental Protection Agency, Cincinnati, Ohio) discussed the possible outcomes of the release of *E. coli* containing recombinant DNA molecules into the aquatic environment and concluded that total reliance cannot be placed on sewage treatment and the natural self-purification capacity of receiving waters to limit potential hazards. While these are realistic barriers to the dissemination of *E. coli* and associated fecal organisms via the water route, they are not infallible because of technological limitations, improper operational practices and system overloading. Finally, M. Starr University of California, Davis) described the numerous genera of gram-negative bacteria found naturally occurring in the soil and on plants. He stated that most of these organisms do not appear to be a reasonable alternative to *E. coli* K-12 as a host for recombinant DNA molecules. Indeed, Starr pointed out that since such genera as *Erwinia*, *Rhizobium* and *Agrobacterium* are known to conjugate with *E. coli*, the potential dissemination of recombinant DNA molecule includes a greater spectrum of microorganisms than just enteric species.

The fourth session of the workshop, chaired by R. Curtiss III (University of Alabama), was concerned with the construction of safer bacterial hosts for DNA cloning. The goals in constructing safer host strains enumerated at the beginning of the session included introduction of mutations that would: (a) preclude colonization in normal ecological niches; (b) preclude cell wall biosynthesis except in specially defined media; (c) cause degradation of genetic information in normal ecological niches; (d) cause vectors to be host-dependent; (e) minimize transmission of recombinant DNA to other strains in normal ecological niches; (f) increase usefulness for recombinant DNA molecule research; and (g) permit monitoring.

Most of the progress in developing safer hosts has been achieved with *E. coli* K-12, although F. Young described a *B. subtilis* strain with a deletion for sporulation genes which readily undergoes autolysis. The strain also has defects in genes for purine and TTP biosynthesis and a mutation conferring a D-alanine requirement can be introduced to cause cell wall biosynthesis to be defective. This strain may be defective in transformation, however, and therefore might be useful only with a phage vector which has yet to be developed and/or discovered.

A. I. Bukhari (Cold Spring Harbor Laboratory) described the use of the *dapD8* mutation in *E. coli* K-12 to block cell wall biosynthesis and another non-reverting mutation which causes sensitivity to bile salts and detergents. The *dapD8* allele is the most stable *dap* point mutation known, although it does revert at frequencies of 10^{-8} to 10^{-6} . The mutation conferring bile salts sensitivity was obtained after Mu-1 infection of an Hfr strain and, although exhibiting the theoretically useful properties of ease of DNA isolation and inability to survive in the intestinal tract, might be due to Mu insertion which would compromise its use for safe strain construction.

Curtiss reported on the work performed by him and his coworkers in constructing and testing numerous strains with different mutations. Survival of strains *in vivo* was tested by feeding rats 10^{10} cells in milk by stomach tube. *Δpur* mutations did not reduce strain titers in feces whereas *ΔthyA*; *ΔthyA drm*; and *ΔthyA dra* mutations gave 10^2 -fold, 10^2 -

fold and 10^4 -fold reductions, respectively, in strain titers in feces. Strains with *ΔthyA* mutations also exhibited thymineless death in *in vitro* tests. Since strains with the *dapD8* allele can revert to *Dap⁺* strains were constructed with both the *dapDs* and *ΔbioH-asd* mutations. These strains have not been observed to revert to *Dap⁺* but can survive passage through the rat intestine and in growth media lacking diaminopimelic acid but containing NaCl and 0.5% usable carbon sources. This survival was due to the production of the mucopolysaccharide, colanic acid, which permits many of the cells to grow and survive as spheroplasts. A *Δgal-chi⁺* mutation (also deletes *latt*, *bio* and *uvrB* genes) was introduced which blocks colanic acid biosynthesis and leads to no detectable survivors in media lacking diaminopimelic acid or following passage through the rat intestine. The *dapD8 ΔbioH-asd Δgal-chi⁺* strains are more readily lysed, transform at higher frequencies and are conjugation-defective in matings with donors possessing conjugative plasmids in the P, W and O incompatibility groups but *Con⁺* as recipients for F, I and T group plasmids when compared to the *dap⁺ gal⁺* parent strain. Strains with *endA* mutations were also observed to exhibit increased transformation frequencies. Attempts to introduce temperature-sensitive *polA* alleles into strains to block replication of ColEI cloning vectors at elevated temperatures and to cause DNA degradation at elevated temperatures in the presence of *recA* and *ΔthyA* alleles often do not have the same properties in the constructed strains as in the strains in which the allele was originally induced. Many mutations causing a *Con⁻* phenotype have been investigated, but many of these revert or do not exhibit a *Con⁻* phenotype in matings with donors possessing conjugative plasmids of the incompatibility groups commonly found in enteric microorganisms. Some *Con⁻* mutants exhibit increased sensitivity to bile salts; thus, the mutant described by Bukhari may also exhibit a *Con⁻* phenotype. All of the strains constructed by the Curtiss group are *SuII⁺* and most have mutations abolishing restriction alone or both restriction and modification. Thus, sufficient information is now known to construct a usable safer *E. coli* K-12 host. Curtiss and collaborators are now introducing the *ΔthyA* and *dna* mutations into their *dapD8 ΔbioH-asd Δgal-chi⁺ uvrB hsr nalA⁺* (for use in monitoring) *Su⁺ λ⁺ φ80⁺* strain to accomplish this objective.

The final session involved a general discussion of some of the major points raised previously in the workshop. There was general agreement at this session that both plasmid-host and phage-host systems have been developed that should meet the criteria of an EK2 system specified by the National Institutes of Health guidelines for research on recombinant DNA molecules. Additional testing is required to confirm the EK2 properties of these available systems, but it is anticipated that these vector-host systems will meet these tests.

Dr. Donald R. Helinski, University of California, San Diego.

Dr. Stanley Falkow, University of Washington.

Dr. Roy Curtiss III, University of Alabama.

Dr. Wacław Szybalski, University of Wisconsin.

APPENDIX D.—SUPPLEMENTARY INFORMATION ON PHYSICAL CONTAINMENT

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I. BIOLOGICAL SAFETY CABINETS

Biological Safety Cabinets suitable for confining operations involving recombinant DNA molecules are described below:

1. *Class I.* A ventilated cabinet for personnel protection only, with an unrecirculated inward flow of air away from the operator. The exhaust air from this cabinet may be filtered through a high-efficiency or high-efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is suitable for research work with the Center for Disease Control (CDC) classes of etiologic agents 1, 2 and 3 where no product protection is required. This cabinet may be used in three operational modes: (i) with an eight-inch high, full-width open front; (ii) with an installed front closure panel (having four, eight-inch diameter openings) without gloves; and (iii) with an installed front closure panel equipped with arm length rubber gloves. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

2. *Class II.* A ventilated cabinet for personnel and product protection having an

open front with inward air flow for personnel protection, and HEPA-filtered recirculated mass air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. Two models of this cabinet are available, Type 1 and Type 2.

(1) *Type 1.* The Type 1 recirculates approximately 70% of the air. The exhaust air from this cabinet may discharge into the laboratory or be diverted out of the laboratory. This cabinet is suitable for CDC classes of etiologic agents 1, 2, and 3. Vapors or gases which are hazardous from a toxic, radioactive, or flammability standpoint should not be used in this cabinet because of the high quantity of recirculated air.

(2) *Type 2.* The Type 2 cabinet recirculates approximately 30% of the air. The exhaust air from this cabinet is normally ducted out of the laboratory through a HEPA filter and, occasionally, an activated charcoal filter depending on the operation. The cabinet may be used with gases or vapors that are hazardous from a toxic, radioactive, or flammability standpoint. However, any consideration of use of such materials should be evaluated carefully from

the standpoint of build-up to dangerous levels and problems of decontamination of the cabinet. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

3. *Class III.* A closed front ventilated cabinet of gas-tight construction providing total protection for personnel and product from contaminants exterior to the cabinet. The cabinet is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is HEPA-filtered. Exhaust air is HEPA-filtered or incinerated to protect the environment. This cabinet, fitted with arm length rubber gloves, provides the highest containment of these three classes of cabinets and is utilized for all activities involving high risk agents (i.e., CDC etiologic agents, class 4). See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

The integrity of any cabinet depends on initial and periodic evaluation to meet established performance tests. Table I outlines the minimum performance required to assure that the cabinets will provide protection of personnel and the environment.

TABLE I.—Biological safety cabinets, safety performance requirements, and specifications, June 1976

Cabinet	Use classification	Performance requirements					Exhaust filter efficiency (percent)
		Face velocity	Exhaust air (cubic feet per minute)		Leak tightness		
		(linear feet per minute)	4-ft hood	6-ft hood			
Class I.....	P1-P3	1-3	75	200	300	Not applicable.....	99.97
Class II, type 1.....	P1-P3	1-3	75	260	400	Gas tight; leak rate <1 by 10 ⁻⁴ cm ³ /s 2-in water gage pressure.	99.97
Class II, type 2.....	P1-P3	1-3	100	250	360	Pressure tight; no air/soap bubble at 2-in water gage pressure.	99.97
Class III.....	P4	4	(3)	(4)	(4)	Gas tight; leak rate <1 by 10 ⁻⁴ cm ³ /s at 3-in water gage pressure.	99.97

¹ For work with recombinant DNA molecules.

² Center for Disease Control (U.S. Public Health Service).

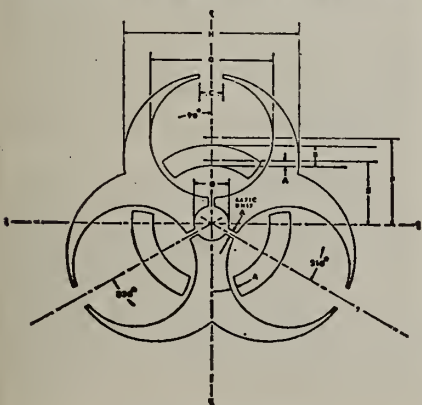
³ Not applicable.

⁴ Based on 1 vol. of air change each 3 min, in the absence of unusual heat or moisture that would require more air changes.

II. UNIVERSAL BIOHAZARD WARNING SYMBOL (1)

The biological hazard warning symbol (biohazard symbol) specified herein shall be used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof which contain or are contaminated with viable hazardous agents.

The biohazard symbol shall be designed and proportioned as illustrated here:



DIMENSION	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
UNITS	1	2 1/2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

The symbol shall be as prominent as practical, and of a size consistent with the size of the equipment or material to which it is affixed, provided the proportions shown above are maintained, and, in any case, that the symbol can be easily seen from as many directions as possible.

Except when circumstances do not permit, the symbol shall be oriented with one of the three open circles pointed up and the other two forming a base.

The symbol color shall be a fluorescent orange or orange-red color.* Background color is optional as long as there is sufficient contrast for the symbol to be clearly defined.



Revised 9-9-66
*Pony-Glo® Fire Orange of the Switzer Brothers, Inc. is cited as an example, not an endorsement.

The biohazard symbol shall be used or displayed only to signify the actual or potential presence of biological hazard.

Appropriate wording may be used in association with the symbol to indicate the nature or identity of the hazard, name of individual responsible for its control, precautionary information, etc., but never should this information be superimposed on the symbol.



ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Hazard identity: _____

Responsible Investigator: _____

In case of emergency call: _____

Daytime phone _____

Home phone _____

Authorization for entrance must be obtained from the Responsible Investigator named above.

III. LABORATORY TECHNIQUES FOR BIOHAZARD CONTROL

A. Pipetting

1. No infectious or toxic materials should be pipetted by mouth (2, 3, 4).

2. No infectious mixtures should be prepared by bubbling expiratory air through a liquid with a pipette (2, 3, 4).

3. No infectious material should be blown out of pipettes (2, 3, 4).

4. Pipettes used for the pipetting of infectious or toxic materials should be plugged with cotton (2, 3, 4).

5. Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion (2, 4). They should not be placed vertically in a cylinder.

6. The pan and pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant (2, 3, 4).

7. Infectious material should not be mixed by alternate suction and expulsion through a pipette (2, 3, 4).

8. Mark-to-mark pipettes are preferable to other types, as they do not require expulsion of the last drop (5).

9. Discharge should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible—not dropped from a height (5).

10. A disinfectant-wetted towel over the immediate work surface is useful in some cases to minimize the splash from accidental droppage (9).

B. Syringes and Needles (9)

1. To lessen the chance of accidental infection, aerosol production or spills, avoid unnecessary use of the syringe and needle. For instance:

(i) Use the needle for parenteral injections but use a blunt needle or a cannula on the syringe for oral or intranasal inoculations.

(ii) Do not use a syringe and needle as a substitute for a pipette in making dilutions of dangerous fluids.

2. Use the syringe and needle in a Biological Safety Cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.

3. Examine glass syringes for chips and cracks, and needles for barbs and plugs.

Note: This should be done prior to sterilization before use.

4. Use needle-locking (Luer-Lok® type) syringes only, and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred.

5. Wear surgical or other type rubber gloves for all manipulations with needles and syringes.

6. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.

7. Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with the proper disinfectant, or into a small bottle of sterile cotton.

8. Do not use the syringe to expel forcefully a stream of infectious fluid into an open vial or tube for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the needle is held below the surface of the fluid in the tube.

9. If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of infectious material to the fingers.

10. When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant. If there is danger of the disinfectant contaminating sensitive experiments, a sterile dry pledget may be used and discarded immediately into disinfectant solution.

11. Inoculate animals with the hand "behind" the needle to avoid punctures.

12. Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.

13. Before and after injection of an animal, swab the site of injection with a disinfectant.

14. Discard syringes into a pan of disinfectant without removing the needle. The syringe first may be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. The filling action clears the needle and dilutes the contents of the syringe. Autoclave syringes and needles in the pan of disinfectant.

15. Use separate pans of disinfectant for disposable and nondisposable syringes and needles to eliminate a sorting problem in the service area.

16. Do not discard syringes and needles into pans containing pipettes or other glassware that must be sorted out from the syringes and needles.

C. Opening Culture Plates, Tubes, Bottles, and Ampoules

1. Plates, tubes and bottles of fungi may release spores in large numbers when opened. Such cultures should be manipulated in a Biological Safety Cabinet (6, 15).

2. In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents, some infections have occurred by this means and are represented in the 80% for which no known act or accident is ascribable (3).

3. Water of syneresis in petri dish cultures is usually infected and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic petri dishes where the lid touches the rim at only three points are less likely to offer this hazard (8, 19).

4. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar.

Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet they should be opened in the Biological Safety Cabinet (8).

5. Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of infected liquid which may collect between the rim and the liner is broken during removal of the closure (8).

6. Dried, infected culture material may also collect at or near the rim or neck of culture tubes and may be dispersed into the air when disturbed (18). Containers of dry powdered hazardous materials (e.g., Class 3 fungal agents in the spore phase of growth) should be opened only in a Biological Safety Cabinet (6, 14).

7. When the neck of an ampoule containing liquid is broken after nicking with a file, the snapping action creates aerosols. The following methods have been recommended:

(i) After nicking the ampoule with a file, wrap the ampoule in disinfectant-wetted cotton before breaking. Wear gloves (2).

(ii) The bottom of the ampoule should be held in several layers of tissue paper to protect the hands, and a file mark made at the neck. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressures. After a few seconds the ampoule should be wrapped in a few layers of tissue and broken along the crack. The tissues and ampoule neck can then be discarded into disinfectant, and the contents of the ampoule removed with a syringe. If the ampoule contains dried cultures, about 0.5 cm³ of broth should be added slowly to avoid blowing dried material out. The contents may then be mixed without bubbling and withdrawn into a culture tube (8).

(iii) The researcher uses an intense, but tiny, gas-oxygen flame and heats the tip of the hard glass ampoule until the expanding internal air pressure blows a bubble. After allowing this to cool, he breaks the bubble while holding it in a large low temperature flame; this immediately incinerates any infectious dust which may come from the ampoule when the glass is broken (16). Preliminary practice with a simulant ampoule of the same type actually in use is necessary to develop a technique that will not cause explosion of the ampoule.

(iv) A simple device has been recommended consisting of a sleeve of rubber tubing into which the ampoule is inserted before it is broken (17, 18).

D. Centrifuging

1. A safety centrifuge cabinet or safety centrifuge cup (3, 7, 8, 14, 22) may be used to house or safeguard all centrifuging of infectious substances. When bench type centrifuges are used in a Biological Safety Cabinet, the glove panel should be in place with the glove ports covered. The centrifuge operation creates air currents that may cause escape of agent from an open cabinet (2, 3, 4, 13).

2. In some situations, in the absence of O-ring cap sealed trunnion cups, specimens can be enclosed in sealed plastic bags before centrifugation (12).

3. Before centrifuging, inspect tubes for cracks, inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter, and carefully remove bits of glass from the rubber cushion (4, 10).

4. A germicidal solution should be added between the tube and trunnion cup to disinfect the materials in case of accidental breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (4, 10).

5. Avoid decanting centrifuge tubes. If you must do so, afterwards wipe off the outer rim

with a disinfectant; otherwise the infectious fluid will spin off as an aerosol (4, 10).

6. Avoid filling the tube to the point that the rim, cap or cotton plug ever becomes wet with culture (4, 10).

7. Screw caps, or caps which fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk, however; if the rim is soiled some fluid will escape down the outside of the tube. Screw-capped bottles may jam in the bucket, and removing them is hazardous. Propping such bottles higher in the bucket with additional rubber buffers is mechanically unsound (8).

8. Kitchen foil is often used to cap centrifuge tubes. This creates more risk than the screw cap. Foil caps often become detached in handling and centrifuging (8).

9. The balancing of buckets is often mismanaged. Care must be taken to ensure that matched sets of trunnions, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts should be included in the procedure; and care must be taken to ensure that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 g of mercury and 20 g of water respectively will balance perfectly on the scales; but their performance in motion is totally different, leading to violent vibration with all its attendant hazards (5).

10. Fill and open centrifuge tubes or trunnion cups in a Biological Safety Cabinet (10).

E. High-Speed Centrifuges (22)

1. In high-speed centrifuges the bowl is connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles the pump and the oil in it will become contaminated. A high efficiency filter should be placed between the centrifuge and the pump (8).

2. High speed rotor heads are prone to metal fatigue, and where there is a chance that they may be used on more than one machine each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning and drying are important to ensure absence of corrosion or other trauma which may lead to creeping cracks. Rubber O-rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance, but are prone to leakage if applied to tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leaking should never occur (5).

3. Cleaning and disinfection of tubes, rotors and other components requires considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly inflammable and prone to shrinkage with age and distortion on boiling, but can be

have as high explosive in an autoclave (5). Large-scale zonal centrifugation requires special attention (11).

F. Blenders, Ultrasonic Disintegrators, Colloid Mills, Ball Mills, Jet Mills, Grinders, Mortar and Pestle.

All these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of infectious materials, the following practices should be observed:

1. Operate blending and cell-disruption and grinding equipment in a Biological Safety Cabinet (9).

2. Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl (9).

3. In the absence of a leak-proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with infected material (9).

4. Sterilize the device and residual infectious contents promptly after use. Use a towel moistened with disinfectant over the top of the blender (9).

5. Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a polypropylene jar to prevent dispersal of glass (8).

6. A new machine, the Colworth Stomacher (England), in which material is homogenized in a plastic bag in a closed container, would appear to be safer than some of the other blenders (8).

7. A heat-sealed flexible plastic film enclosure for a grinder or blender can be used, but it must be opened in a Biological Safety Cabinet (7).

8. Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product (7).

9. Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

10. Clinical or other laboratories handling human blood should be aware of the aerosols produced by the microhaematocrit centrifuge, the autoanalyzer stirrer, and the microtometer, inasmuch as it seems that airborne transmission of infectious hepatitis may occur in the laboratory (20).

G. Miscellaneous Precautions and Recommendations

1. Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended (4, 10).

2. Deepfreeze, liquid nitrogen, and dry ice chests and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deepfreezes should be properly labelled. Security measures should be commensurate with the hazards (4, 10, 21).

3. Freeze-dried culture ampoules should always be opened in a Biological Safety Cabinet. The ampoule should be wrapped in a disinfectant-soaked swab before breaking it open to minimize the risk of cutting the hands, and to a lesser extent of releasing aerosol of dried material. Whenever possible, ampoules should be filled with dry nitrogen after freeze-drying, thus avoiding implosion that may occur during the sealing as well as opening of evacuated ampoules. The whole process of freeze-drying itself should be performed in a Biological Safety Cabinet. Filtration of the effluent air from the vacuum pump is desirable either up (preferably), or down stream of the pump (5).

4. Ensure that all virulent fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leak-proof containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel (4, 10).

5. All inoculated petri plates or other inoculated solid media should be transported and incubated in leak-proof pans or leak-proof containers (4, 10).

6. Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proved their sterility (4, 10).

7. Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

8. No person should work alone on an extremely hazardous operation (4, 10).

IV. PERSONAL HYGIENE, HABITS, AND PRACTICES

Personal hygienic practices in the laboratory are directed, in most part, toward the prevention of occupationally acquired physical injury or disease. To a less obvious extent, they can raise the quality of the laboratory work by reducing the possibilities for contamination of experimental materials. The reasons for many of the recommended precautions and practices are obvious, but, in some instances, amplification will permit a better review of the applicability to any one specific laboratory.

Consequently, what might be forbidden in one laboratory might be only discouraged in another, and be permissible in a third. Nevertheless, adherence to safe practices that become habitual, even when seemingly not essential, provides a margin of safety in situations where the hazard is unrecognized. The history of occupational injury is replete with examples of hazards unrecognized until too late. The following guidelines, recommendations, and comments are presented with this in mind:

1. Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory (5, 10).

2. Foot-operated drinking fountains should be the sole source of water for drinking by human occupants of the laboratory (27).

3. Smoking is not permitted in the laboratory or animal quarters. Cigarettes, pipes, and tobacco will be kept only in clean areas (5, 10, 26).

4. Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toilet supplies, and cosmetics are permissible only in clean change rooms or other clean areas, and should never be used until after showering or thorough washing of the face and hands (27).

5. A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a face mask or respirator when the work requires respiratory protection (10, 27, 31).

6. Develop the habit of keeping hands away from mouth, nose, eyes, face, and hair. This may prevent self-inoculation (10, 27).

7. For product protection, persons with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirements in hospital operating rooms and in the manufacture of biological pharmaceutical products. A head cover also will protect the hair from fluid splashes, from swinging into Bunsen flames and petri dishes, and will reduce facial contamination caused by habitual repetitive manual adjustment of the hair (5).

8. Long-flowing hair and loose-flapping clothing are dangerous in the presence of open flame or moving machinery. Rings and wrist watches also are a mechanical hazard during operation of some types of machines (5, 10).

9. Contact lenses do not provide eye protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Cautic chemicals trapped in this space cannot be washed off the surface of the cornea. If the material in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible, to remove the lens. For this reason, contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles, or plastic face masks are also worn to provide full protection. It is the responsibility of supervisors to identify employees who wear contact lenses (25, 26).

10. Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be kept elsewhere (25).

11. Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds, and other potential microbial contaminants of biological experimental materials (25).

12. Books and journals returnable to the institutional library should be used only in the clean areas as much as possible (10, 27).

13. When change rooms with showers are provided, the employer should furnish skin lotion (27).

14. When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap (8, 27).

15. Personal cloth handkerchiefs should not be used in the laboratory. Cleansing tissue should be available instead.

16. Hand washing for personal protection:

- (i) This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.

- (ii) Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wrist watch discourages adequate washing of the wrist (10, 25).

- (iii) Hands should be washed after removing soiled protective clothing, before leaving laboratory area, before eating, and before smoking. The provision of hand cream by the employer encourages these practices (5, 8, 10).

- (iv) A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.

17. Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infective material unless the injured area is completely protected (8, 25).

18. Persons vaccinated for smallpox may be shedders of vaccinia virus during the phase of cutaneous reaction. Therefore, vaccination requires permission of the appropriate supervisor, because two weeks' absence may be necessary before returning to work with

normal cell cultures or with susceptible animals, especially the normal mouse colony (25).

19. The surgeon's mask of gauze or filter paper is of little value for personal respiratory protection (29). It is designed to prevent escape of droplets from the nose or mouth (23G). If biohazards demand respiratory protection, then nothing but a full face respirator or ventilated hood will suffice. A half-mask respirator does not protect the eyes, which are an unevaluated avenue of infection through the conjunctiva and the nasolacrimal duct (5, 8).

20. Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or supplies, and outside air is a complication that may affect or invalidate the results of an experiment. The human sources of this contamination are evaluated as follows:

(i) Sneezing, coughing and talking (23A, 24A). Sneezing, variously reported to generate as many 32,000 or 1,000,000 droplets below 100 microns in diameter; coughing, which produces fewer and larger droplets; and talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized. As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission or airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.

(ii) Dispersal of bacteria from human skin. There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject. For instance, in one study, the number varied from 6,000 to 60,000 per minute (23C). These bacteria were released on skin scales which were of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test (23D). Dispersal of skin bacteria was several times greater from below the waist than from upper parts of the body (24D). Effective reduction is accomplished by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as a bellows that disperses air carrying skin scales laden with bacteria (23B). Such clothing sometimes is too warm to work in. It was found that a significant reduction in dispersal of bacteria occurred with the wearing of close-fitting and closely-woven underpants beneath the usual laboratory clothing (23D). The purpose of this summary is to alert laboratory personnel to the existence of this source of contamination (9).

(iii) Prolific dispersal of bacteria occurs from infected abrasions, small pustules, boils, and skin disease (23F, 24B). Washing the lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air. Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair (23E). This point may be useful in investigating the source of staphylococcal contamination of cell lines.

(iv) Footwear. In moderate and high risk situations, shoes reserved for only laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. However, in experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can reduce the microbial load brought into the laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself (30).

V. CARE AND USE OF LABORATORY ANIMALS (10,32-37)

A. Care and Handling

1. Special attention must be given to the humane treatment of all laboratory animals in accordance with the Animal Welfare Act of 1970. The implementing rules and regulations appear in the Code of Federal Regulations (CFR) Title 9, Chapter 1, Subchapter A, parts 1, 2, 3. Recommended provisions and practices that meet the requirements of the Act have been published by the U.S. Public Health Service (32).

2. There are specific minimum requirements (33) concerning the caging, feeding, watering, and sanitation for dogs, cats, guinea pigs, hamsters, rabbits, and non-human primates. To meet these requirements, the animal room supervisor must have a copy of 9 CFR Chapter 1, Subchapter A, Parts 1, 2, 3.

3. Each laboratory should establish procedures to ensure the use of animals that are free of disease prejudicial to the proposed experiments and free from carriers of disease or vectors, such as ectoparasites, which endanger other experimental animals or personnel (10).

B. Cages Housing Infected Animals (10)

1. Careful handling procedures should be employed to minimize the dissemination of dust from cage refuse and animals.

2. Cages should be sterilized by autoclaving. Refuse, bowls and watering devices should remain in the cage during sterilization.

3. All watering devices should be of the "non-drip" type.

4. Cages should be examined each morning and at each feeding time so that dead animals can be removed.

5. Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should NEVER be placed in the cage to move any object therein.

6. When animals are to be injected with biohazardous material, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeons gloves. Animals should be properly restrained to avoid accidents that might result in disseminating biohazardous material, as well as to prevent injury to the animal and to personnel.

7. Animals exposed to biohazardous aerosols should be housed in ventilated cages, in gas-tight cabinet systems, or in rooms designed for protection of personnel by use of ventilated suits.

8. Animals inoculated by means other than by aerosols should be housed in equipment suitable for the level of risk involved.

9. Infected animals to be transferred between buildings should be placed in ventilated cages or other aerosol-proof containers.

10. The oversize canine teeth of large monkeys present a particular biting hazard; these are important in the potential transmission of naturally-occurring, and very dangerous, monkey virus infections. Such teeth should be blunted or surgically removed by a veterinarian.

11. Presently available epidemiological evidence indicates that infectious hepatitis may be transmitted from nonhuman primates (typically chimpanzees) to man. Newly imported animals may be naturally infected with this disease, and persons in close contact with such animals may become infected. After six months residence in this country, chimpanzees apparently no longer transmit the disease. A record should be maintained for each newly imported animal. A sign should be posted at rooms housing these animals to warn that the animals are potentially infectious.

C. General Guidelines that Apply to Animal Room Maintenance (10)

1. Doors to animal rooms should be kept closed at all times except for necessary entrance and exit.

2. Unauthorized persons should not be permitted to enter animal rooms.

3. A container of disinfectant should be kept in each animal room for disinfecting gloves and hands, and for general decontamination, even though no infectious animals are present. Hands, floors, walls, and cage racks should be washed with an approved disinfectant at the recommended strength as frequently as the supervisor directs.

4. Floor drains in animal rooms, as well as floor drains throughout the building should be flooded with water or disinfectant periodically to prevent backup of sewer gases.

5. Shavings or other refuse on floors should not be washed down the floor drain because such refuse clogs the sewer lines.

6. An insect and rodent control program should be maintained in all animal rooms and in animal food storage areas.

7. Special care should be taken to prevent live animals, especially mice, from finding their way into disposable trash.

D. Necropsy Rules for Infected Animals (10)

1. Necropsy of infected animals should be carried out by trained personnel in Biological Safety Cabinets with the hinged glass panel down. The glove port panel with or without attached gloves, and a respirator should be used at the discretion of the supervisor.

2. Surgeons gowns should be worn over laboratory clothing during necropsies.

3. Rubber gloves should be worn when performing necropsies.

4. The fur of the animal should be wetted with a suitable disinfectant.

5. Small animals should be pinned down or fastened on wood or metal in a metal tray.

6. Upon completion of necropsy, all potentially biohazardous material should be placed in suitable containers and sterilized immediately.

7. Contaminated instruments should be placed in a horizontal bath containing a suitable disinfectant.

8. The inside of the Biological Safety Cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide.

9. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, preparatory to sterilization.

10. Dead animals should be placed in proper leak-proof containers, autoclaved and properly tagged before being placed outside for removal and incineration.

VI. DECONTAMINATION AND DISPOSAL (7, 10, 38-42)

A. Introduction

Available data on the efficacy of various decontaminants for etiologic agents indicate that no major surprises will be forthcoming regarding the susceptibility of organisms containing recombinant DNA molecules. In the absence of adequate information, tests to determine the efficacy of candidate decontaminants should be conducted with the specific agent of interest. The goal of decontamination is not only the protection of personnel and the environment from exposure to infectious agents, but also the prevention of contamination of experimental materials by a variable, persistent, and unwanted background of microorganisms. This additional factor should be considered in selecting decontamination materials and methods.

B. Decontamination Methods

Physical and chemical means of decontamination fall into four main categories: Heat; Liquid Decontaminants; Vapors and Gases; and UV Radiation.

1. *Heat.* The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility. Dry heat at 160 to 170°C for periods to 2 to 4 hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat in the decontamination of microorganisms and also serves as an efficient means for disposal.

2. *Liquid Decontaminants.* In general, the liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems.

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. Unfortunately, the more active the decontaminant the more likely it is that the decontaminant will possess undesirable characteristics, such as the possession of corrosive properties. None is equally useful or effective under all conditions.

3. *Vapors and Gases.* A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating: (i) Biological Safety Cabinets and associated effluent air-handling systems and air filters; (ii) bulky or stationary equipment that resists penetration by liquid surface decontaminants; (iii) instruments and optics that might be damaged by other decontamination methods; and (iv) rooms and buildings and associated air-handling systems.

4. *Radiation.* The usefulness of ultraviolet (UV) irradiation as a decontaminant is limited by its low penetrating power. No information is available regarding the effectiveness of UV irradiation for decontaminating microorganisms containing recombinant DNA molecules. Dependence on UV must be based on the results of experiments imitating particular anticipated environmental conditions and applications. Ultraviolet light is generally of limited application and is primarily useful in air locks and animal holding areas for controlling low levels of airborne contaminants.

No one procedure or material will solve all decontamination problems. The only

method of assuring the efficacy of selected methodologies is to critically examine the results obtained in practical tests with the microorganism(s) of interest.

C. Laboratory Spills

A troublesome problem that may occur in the laboratory is the decontamination of an overt biological spill. The occurrence of a spill poses less of a problem if it occurs in a Biological Safety Cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid decontaminant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone but gaseous decontaminants would be required to rid the interior sections of the cabinet of contaminants. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

The greater problem arises if the incident occurs in the open laboratory. All laboratory protocols should be designed to prevent such occurrences. The first action in the event of an overt laboratory spill is evacuation of the affected area to minimize the exposure of personnel involved. Next, the spill area must be isolated to prevent exposure of personnel and experimental materials beyond those involved in the immediate area of the spill. The procedures adopted must be rapidly effective and must not create additional aerosol or foster mechanical transfer of materials to unaffected areas. Personnel carrying out the procedures must be provided with protective clothing and equipment, including respiratory protection. Consideration must be given to the safe disposal of all materials and liquids resulting from cleanup procedures. Reentry of personnel to the area should be avoided until it can be reasonably established that the area has been effectively decontaminated. Further specific details are provided in Section VIII.

D. Disposal

Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which decontamination constitutes the introductory phase of disposal. All materials and equipment used in research on recombinant DNA molecules will ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or on-site destruction. The remainder will be recycled for use either within the same laboratory or in other laboratories that may or may not engage in DNA recombinant research. Examples of the latter that immediately come to mind are: reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of the word, rather than in the restrictive sense of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated by an approved procedure?
2. If not, have the objects or materials been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?
3. Does disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to personnel either:

(i) Those carrying out the immediate disposal procedures or

(ii) Those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solid, and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be decontaminated prior to disposal. It is most evident that a significant portion of this problem can be eliminated if the kinds of materials initially entering the laboratory are reduced. In any case, and wherever possible, materials not essential to the research should be retained in the nonresearch areas for disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton-cages for transport of animals, and large carboys or tanks of fluids which can be left outside and drawn from as required. Reduction of this bulk will free autoclaves and other decontamination and disposal processes within the laboratory for the more rapid and efficient handling of materials known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by requiring that each investigator decontaminate all contaminated materials not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories where the mass of materials for disposal becomes much greater and sterilization and decontamination bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a case can be made for establishing a positive method of designating the state of materials to be disposed of. This may consist of a tagging system stating that the materials are either sterile or contaminated.

Disposal of materials from the laboratory and animal holding areas will be required for research projects ranging in size from an individual researcher to those involving large numbers of researchers of many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner and with as little thought as household wastes. Selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of dissimilar nature will be common in laboratories studying recombinant DNA molecules. Examples are combinations of common flammable solvents, chemical carcinogens, radioactive isotopes, and concentrated viruses or nucleic acids. These may require input from a number of disciplines in arriving at the most practical approach for their decontamination.

E. Characteristics of Chemical Decontaminants in Common Use in Laboratory Operations

Every person actively working with viable microorganisms, no matter how remote the field of specialization, will, from time to time, find it necessary to decontaminate by chemical methods work areas and materials, equipment, and specialized instruments. Chemical decontamination is necessary because the use of pressurized steam, the most rapid and reliable method of sterilization, is not normally feasible for decontaminating large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture often damage delicate instruments,

particularly those having complex optical and electronic components.

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, and liquid concentrates. These may be added to tap water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperatures are useful as space-penetrating decontaminants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

1. Coagulation and denaturation of protein.
2. Lysis.
3. Binding to enzymes, or inactivation of an essential enzyme by either oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay systems employed.

There are dozens of decontaminants available under a wide variety of trade names. In general, these decontaminants can be classified as halogens, acids or alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics. For example, peracetic acid is a fast-acting, universal decontaminant. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding decontaminant that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are probably the second most active group of decontaminants. Chlorine, iodine, bromine, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. These decontaminants are effective over a wide range of temperatures. In fact, chlorine has been shown to be effective at -40 F. (On the other hand, phenols and formaldehyde have high temperature coefficients). The halogens have several undesirable features. They readily combine with protein, so that an excess of the halogen must be used if proteins are present. Also, the halogens are relatively unstable so that fresh solutions must be prepared at frequent intervals. Finally, the halogens corrode metals. A number of manufacturers of decontaminants have treated the halogens to remove some of the undesirable features. For example, sodium hypochlorite reacts with p-toluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are stable, non-toxic, odorless, and relatively noncorrosive to metals. However, the halogens are highly reactive elements, and, because they are reactive they are good germicides. When a halogen acts as a decontaminant, free halogen is the effective agent. Raising the pH or combining the halogen with other compounds to decrease the corrosive effect will also decrease

the germicidal power. A trade-off situation occurs.

Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If one places an item in a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful, and a decontaminant should have, and most do have, incorporated surface-active agents.

F. Properties of Some Common Decontaminants

1. *Alcohol.* Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective decontaminants against lipid-containing viruses.

2. *Ether and Chloroform.* These compounds are not ordinarily used as decontaminants, but they do demonstrate the fact that lipid-containing viruses are inactivated by these organic solvents, whereas non-lipid-containing viruses are quite resistant.

3. *Formaldehyde.* Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from formaldehyde solution is an effective space decontaminant for decontaminating rooms or buildings, but in the vapor state with water it tends to polymerize out on surfaces to form paraformaldehyde, which is persistent and unpleasant. Formaldehyde gas can be liberated by heating paraformaldehyde to depolymerize it. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

4. *Phenol.* Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

5. *Quaternary Ammonium Compounds or Quats.* After 30 years of testing and use, there is still a considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats have the advantages of being nontoxic, odorless, nonstaining, noncorrosive to metals, stable, and inexpensive.

6. *Chlorine.* This halogen is a universal decontaminant active against all micro-

organisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them to 1 to 100, the solution will contain 525 ppm of available chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

7. *Iodine.* The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water giving 25 ppm of available iodine to 3 oz. in 5 gal. giving 775 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 or 10% in 50% ethyl alcohol (a reasonably good decontaminant itself) which will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

G. Vapors and Gases

The use of formaldehyde as a vapor or gas has already been discussed. Other chemical decontaminants which have been used this way included ethylene oxide, peracetic acid, beta-propiolactone (BPL), methyl bromide, and ethylene amine. When these can be used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can be obtained. Residues from ethylene oxide must be removed by aeration; but otherwise it is convenient to use, versatile, and noncorrosive. Peracetic acid is corrosive for metals and rubber. BPL in the vapor form acts rapidly against bacteria, rickettsiae, and viruses. It has a half-life of 3.5 hours when mixed with water, is easily neutralized with water, and lends itself to removal by aeration. The National Institutes of Health does not recommend BPL as a decontaminant because it has been identified as a suspect carcinogen.

H. Residual Action of Decontaminants

As noted in the preceding discussion of decontaminant properties, many of the chemical decontaminants often have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize laboratory shoes can leave residues which cause skin irritation. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by the decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area decontamination with gases or vapors, it may be necessary to protect new and used clean items by removing them from the area or by enclosing

them in gastight bags or by insuring adequate aeration following decontamination.

1. Selecting Chemical Decontaminants for Research on Recombinant DNA Molecules

No single chemical decontaminant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

1. What is the target microorganism(s)?
2. What decontaminants in what form are known to, or can be expected to, inactivate the target microorganism(s)?
3. What degree of inactivation is required?
4. In what medium is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?
5. What is the highest concentration of cells anticipated to be encountered?
6. Can the decontaminant either as an aqueous solution, a vapor, or a gas reasonably be expected to contact the microorganisms, and can effective duration of contact be maintained?
7. What restrictions apply with respect to compatibility of materials?
8. Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations or infectious agents usually have titers grossly in excess of those

normally observed in nature. The decontamination of these high-titer materials presents certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid-containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

A decontaminant selected on the basis of its effectiveness against microorganisms on any range of the resistance scale will be effective against microorganisms lower on the scale. Therefore, if decontaminants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other microorganisms generated by laboratory operations, even in high concentrations, would also be inactivated.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acid. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Because of innate differences in the chemistry of RNA and DNA the effectiveness of a decontaminant for one cannot be extrapolated to the other. For example, RNA molecules are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2' position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

Table II summarizes pertinent characteristics and potential applications for several categories of chemical decontaminants most likely to be used in the biological laboratory. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstrooms. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the decontaminants should be conclusively determined by individual investigators. It is readily evident that each of the decontaminants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of decontaminants.

TABLE II

SUMMARY OF PRACTICAL DECONTAMINANTS FOR USE IN THE LABORATORY

DECONTAMINANTS		PRACTICAL REQUIREMENTS				INACTIVATES				IMPORTANT CHARACTERISTICS										POTENTIAL APPLICATION										PREPARATORY DECONTAMINANTS EXAMPLES						
		CONCENTRATION	CONTACT TIME	ACTIVE INGREDIENT	FORM	WATER SOLUBLE	VEG. BACT.	LIPID VIRUS	HYDROLIC VIRUS	BACT. SPORES	EFFECTIVE SELF LIFE	COMBUSTIBLE	FLAMMABLE	EXPLOSION POTENTIAL	RESIDUE	INACTIVATES BY ORGANIC MATTER	LESS COMPATIBLE	ELECTRONIC COMPATIBLE	SELF IRRADIANT	EYE IRRITANT	RESPIRATORY IRRITANT	TOXIC	MOIST SURFACES	DIRTY GLASSWARE	LARGE AREA DECON.	WATER WASHING SYSTEMS	PORTABLE EQUIP. SURFACE DECON.	PORTABLE EQUIP. SURFACE DECON.	STATIONARY EQUIP. SURFACE DECON.		STATIONARY EQUIP. SURFACE DECON.	LIQUIDS FOR DISINFECT	LIQUIDS FOR DISINFECT	LIQUIDS FOR DISINFECT	LIQUIDS FOR DISINFECT	
LIQUIDS	Quat. Ammon. CPB	2%	10	H.E.	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A-33, CIO, DIO-SAC, RI-PCR, RECO-QUAT	
	Phenolic CPB	2%	10	H.E.	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	RI-PCR, RI-PCR, RI-PCR, O-PCR		
	Chlorine CPB	2%	10	30	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	CHLORINE T, CHLORINE, PURGE		
	Iodophor	2%	10	30	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	RI-SAC, IOPH, ITEROLINE, WACORINE		
	Alcohol, Ethyl	95%	10	H.E.	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Alcohol, Isopropyl	95%	10	H.E.	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Formaldehyde	8%	10	30	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	FORMAL	
	Glutaraldehyde	2%	10	30	LIQ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	GLUTAR	
GAS	Ethylene Oxide	450/2	60	60	37	30	+	+	+	+	H.A.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	CIDEX
	Paraformaldehyde	30/10	60	60	23	60	+	+	+	+	H.B.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	CANOLINE, CYCLOLINE, STERILIZER	

- H.A. = Not applicable; H.E. = Not effective.
 1. Variable results dependent on virus.
 2. Neither flammable or explosive in 90% O₂ or flammable hydrocarbon use form.
 3. At concentrations of 7 to 75% by volume in air, solid-response to open flame.
 4. Protected from light and air.
 5. By skin or mouth or both - refer to manufacturer's literature and/or MSD Index.
 6. Space limitations preclude listing all products available. Individual listings (or omissions) do not imply endorsement or rejection of any product by the NIH.
 7. Refer to microscope and camera lenses.

VII. HOUSEKEEPING

A. Introduction

Well-defined housekeeping procedures and schedules are essential in reducing the risks of working with etiologic agents and in protecting the integrity of the research program. This is particularly true in the biological laboratory operating under less than total containment concepts and in all areas used

for the housing of animals, whether or not they have been intentionally infected. A well-conceived and well-executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that pro-

motes the efficient use of decontaminants in the event of the inadvertent release of a harmful agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, an appreciation of the nature and sources of biological contamination.

Housekeeping is an omnibus term that can be interpreted as broadly or as narrowly as one chooses. It can be seen that many of the

procedures found under special headings, such as decontamination, disposal, and animal care, are, in reality, specific instructions for safely accomplishing otherwise routine housekeeping chores. In these safety suggestions for research on recombinant DNA molecules, it has been elected to address specifically only tasks of a janitorial nature under the subject of housekeeping.

The objectives of housekeeping in the biological laboratory are to:

1. Provide an orderly work area conducive to the accomplishment of the research program.
2. Provide work areas devoid of physical hazards.
3. Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems being researched.
4. Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
5. Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that (i) may harbor microorganisms that are a potential threat to the integrity of the biological systems under investigation, (ii) may enhance the survival of microorganisms inadvertently released in experimental procedures, (iii) may retard penetration of decontaminants, (iv) may be transferable from one area to another on clothing and shoes, (v) may, with sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement, and (vi) may cause allergenic sensitization of personnel, e.g., to animal danders.

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other areas in the laboratory have the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological complex are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following checklist outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the biological laboratory complex must be viewed.

Administration Areas
Aisles
Animal Food Storage
Animal Bedding Storage

Biological Safety Cabinets
Bench Tops and Other Work Surfaces
Ceilings
Change Rooms
Cleaning Solution Disposal
Cages and Cage Racks
Dry Ice Chests
Deep Freeze Chests
Entry and Exit Ways
Equipment Storage
Floors
Glassware
General Laboratory Equipment Cleanup
Hallways
Incubators
Instruments
Insect and Rodent Control
Light Fixtures
Mechanical Equipment Areas
Mops
Pipes—Wall and Ceiling Hung
Refrigerators
Showers
Supply Storage
UV Lamps
Vacuum Cleaners
Waste Accumulations
Waste Water Disposal
Others

Housekeeping in the laboratory is one of the avenues that leads to accomplishing the research program safely. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research program and special hazards of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. He should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by an alien cleanup crew, delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements, and the location of concentrated biological preparations and contaminated equipment used in their preparation and application will be known.

B. Floor Care

Avoidance of dry sweeping and dusting will reduce the formation of nonspecific environmental aerosols. Wet mopping or vacuum cleaning with a high-efficiency particulate air (HEPA) filter on the exhaust is recommended.

Careful consideration must be given to design and quality in the selection of cleaning equipment and materials and in their use to prevent the substitution of one hazard for another.

In the absence of overt hazardous spills, the cleaning process commonly will consist of an initial vacuuming to remove all gross particulate matter and a follow-up wet mopping with a solution of chemical decontaminant containing a detergent. Depending on the nature of the surfaces to be cleaned and availability of floor drains, removal of residual cleaning solutions can be accomplished by a number of methods. Among these are: pickup with a partially dry mop, pickup with a wet vacuum that has an adequately filtered exhaust, or removal to a convenient floor drain by use of a floor squeegee.

After cleaning up a spill of infected material, the residual solution should not be discharged to a sanitary sewer until it has

been autoclaved or given further chemical treatment, such as by the addition of sodium hypochlorite sufficient to provide a final concentration of 500 ppm chlorine. Most household bleaches are marketed with a chlorine content of 5.25%. These in a final dilution of 1:100, yield 525 ppm of available chlorine. After allowing a contact time of 15 minutes, these solutions may be flushed down any available drain. Chlorine solutions in these high concentrations may be too corrosive for general application to floors and equipment. In any event, if solutions are used in this way, after the contact time the area should be rinsed with water.

C. Dry Sweeping

While it is recommended that dry sweeping be minimized, this may be the only method available or practicable under certain circumstances. In such cases, sweeping compounds used with push brooms and dry-dust mop heads treated to suppress aerosolization of dust should be used.

Sweeping compounds available from the usual janitorial supply firms fall in three categories:

Wax-based compounds used on vinyl floors and waxed floor coverings.

Oil-based compounds for concrete floors. Oil-based compounds with abrasives (such as sand) to achieve a dry scouring action where much soil is present.

Dry-dust mop heads can be purchased as treated disposable units or as reusable, washable heads that must be treated with appropriate sprays or by other means to improve their dust-capturing property.

D. Vacuum Cleaning

In the absence of a HEPA filter on the exhaust, the usual wet and dry industrial-type vacuum cleaner is a potent aerosol generator. The HEPA-filtered exhaust used in conjunction with a well-sealed vacuum unit, however, can negate this factor because of its ability to pass large volumes of exhaust air while retaining particles with a minimum efficiency of 99.97%. Wet and dry units incorporating a HEPA filter on the exhaust are available from a number of manufacturers.

There are no particular requirements with respect to the manner in which the dry vacuuming is accomplished other than to emphasize that the objective is to remove all debris and particulate matter. The manufacturer's directions adequately detail the frequency of bag changes, filter changes, and mechanical adjustments.

Dry material vacuum-collected during these floor-cleaning activities is potentially contaminated, but the nature of the risk is probably greater to the experiment than to the experimenter. It is wise to effect bag and filter changes and to clean out collection tanks in a manner that will avoid or minimize aerosolizing the contents of the vacuum cleaner.

A vacuum machine that collects debris in a disposable bag is preferable to machines that collect the major debris in a tank and on an exposed primary filter. Even though it may serve as a primary filter, the disposable bag must be removed with caution. A bellows effect may pump dust out of the bag if its intake opening is not sealed before moving it to a plastic bag for transfer out of the area. In any event, the outer surface of the disposable bag will probably bear some dust contamination, which also may occur on inner surfaces of the machine.

To avoid contaminating experimental materials, the emptying of vacuum collection tanks and changing of bags and filters are best done away from the immediate laboratory area, for example, in a small area that can be easily cleaned afterwards. The use of heavy rubber gloves is recommended when

removing wastes from tanks in case broken glass is present. After making the filter changes, all external surfaces of the immediate work area and the equipment should be wiped with a cloth moistened in decontaminant. The operator might plan for a change of laboratory clothing afterwards so as to minimize carrying contamination into other areas of the laboratory.

Avoid use of dry vacuum cleaning equipment in work with high risk agents in the open laboratory. Should it be necessary to use it, it is recommended that gaseous sterilization be used to minimize aerosolization of microorganisms before waste is emptied from the vacuum container. Because complete penetration of sterilizing gases into the collected dry dust may be a problem, all wastes should be placed in a plastic bag, which then is tightly closed and incinerated or disposed of in an approved manner.

When dry vacuum cleaning equipment has been used within a gastight safety cabinet system, it can be treated in an attached double-door carboxyclave (an autoclave equipped with an ethylene oxide gas sterilization system) to allow for removal and emptying of the collection tank.

If a wet vacuum is to be used for pickup of the detergent-germicide solution from the floor, the manufacturer's recommendations on filter life should be followed. In addition, the operation of the vacuum should be closely observed for evidence of operating changing indicating restricted airflow or, conversely, increased flow indicating filter failure. Liquids collected in the vacuum cleaner after floor mopping will contain decontaminant material. These liquids may be poured down a convenient floor drain, except in the case of cleanup wastes from an overt spill. The collected liquid should then be autoclaved or treated with chlorine solution before disposal.

Provisions should be made for regular decontamination of the entire vacuum cleaner with formaldehyde gas or vapor, or ethylene oxide. This should be done after use if the vacuum is used in any manner for cleanup of overt spills of infectious material.

E. Selection of a Cleaning Solution

The selection of a detergent-decontaminant combination for routine cleaning of the laboratory complex should be based on the requirements of the area of greatest potential for contamination by the widest spectrum of microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms may well include fungi, viruses, and the vegetative and spore forms of bacteria. A decontaminating solution for such a range of microorganisms would, however, be expensive and excessively corrosive for routine use. Except in those rare instances where it can be assumed that pathogenic spores are being shed by laboratory animals, the risks from the spores are more likely to affect the experiments than the personnel. The spores tend to be associated with organic debris from bedding and food, thus offering potential for removal or at least a large initial reduction in their numbers by vacuum cleaning. A wide range of cleaning solutions that are mildly sporicidal, reasonably residual, and are not destructive to the physical plant are available. Phenol derivatives in combination with a detergent have these characteristics and have been selected for routine use in a number of research facilities. There are numerous detergent-phenolic combinations available on the market. The phenols are one type of a broad spectrum of biocidal substances that

includes the mercurials, quaternary ammonium compounds, chloride compounds, iodophores, alcohols, formaldehyde, glutaraldehyde, and combinations of alcohol with either iodine or formaldehyde. These have been discussed in Section VI.

The laboratory supervisor should make a selection from those types most readily available which meet the general criteria of effectiveness, residual properties, and low corrosiveness.

F. Wet Mopping—Two-Bucket Method

Wet mopping of floors in laboratory and animal care areas is, from a safety standpoint, most conveniently and efficiently accomplished using a two-bucket system. The principal feature of such a system is that fresh detergent-decontaminant solution is always applied to the floor from one bucket, while all spent cleaning solution wrung from the mop is collected in the second bucket. Compact dolly-mounted double-bucket units with foot-operated wringers are available from most janitorial supply houses. A freshly laundered mop head of the cotton string type should be used daily. This requires that a mop with removable head be provided as opposed to a fixed-head type. In practice, the mop is saturated with fresh solution, very lightly wrung into the second bucket and applied to the floor using a figure eight motion of the mop head. After every four or five strokes, the mop head is turned over and the process continued until an area of approximately 100 ft² has been covered. After allowing a contact time of five minutes, the solution is removed with either a wet vacuum cleaner with HEPA-filtered exhaust or with the wrung-out mop. The mopping is continued in 100 ft² increments until the total floor area has been covered. Floor-cleaning procedures are most effectively completed after the majority of the work force has departed and should progress from areas of least potential contamination to those of greatest potential. Before a mop head is sent to a laundry, it should be autoclaved. Spent cleaning fluids are disposed of by flushing down the drain.

If the cleanup follows an overt spill of infectious material, the spent cleaning solution, after removal from the floor, should be autoclaved or treated with chlorine solution. Chlorine (as household bleach) should be added to give 500 ppm and held for a contact time of 15 minutes before dumping in the sanitary sewer.

G. Alternative Floor Cleaning Method for Animal Care Areas and Areas with Monolithic Floors

The absence of permanently placed laboratory benches and fixed equipment, coupled with the mobility of modern cage racks, makes possible alternate floor-cleaning procedures in animal care facilities. As in all considerations of methodologies in biomedical laboratory facilities, it is necessary to assess the compatibility of procedures and facilities from the hazard point of view. The alternative floor-cleaning procedure to be discussed requires that floors are completely sealed or of monolithic construction so that liquid leakage to adjacent areas does not occur and that floor drains or wet vacuum cleaners are available.

Subsequent to the removal of all debris by dry vacuum, move the cage racks to one side of the room. Cover the floor of the remaining cleared portion of the room with detergent-decontaminant solution applied at a rate of approximately one gallon per 144 ft² from a one-gallon tank sprayer, using a setting of the nozzle which will cause the solution to flow on and not create a spray. The nozzle is placed close to the floor. Allow a fifteen-minute contact period; then push the clean-

ing solution to the floor drain with a large floor squeegee or pick it up with a wet vacuum. Allow the flow to air dry; move the cage racks into the cleaned area, and repeat the process for the remaining floor area. Floor drains in these areas should be rim-flush, at least six inches in diameter, and fitted with a screen or porous trap bucket to catch large debris that escapes the initial dry cleaning. Such screens and baskets should be emptied after treatment with a decontaminant. If space utilization does not require frequent floor washdown, pour a half-gallon of detergent-decontaminant solution into the drain each week to keep the trap in the waste line filled against backup of sewer gases.

VIII. CLEAN-UP OF BIOHAZARDOUS SPILLS

(8, 9, 10)

A. Biohazards Spill in a Biological Safety Cabinet

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

1. Spray or wipe walls, work surfaces, and equipment with a 2% solution of an iodophor-decontaminant (Wescodyne or equivalent). A decontaminant detergent has the advantage of detergent activity, which is important because extraneous organic substances frequently interfere with the reaction between the microorganisms and the active agent of the decontaminant. Operator should wear gloves during this procedure.

2. Flood the top work surface tray, and, if a Class II cabinet, the drain pans and catch basins below the work surface, with a decontaminant and allow to stand 10-15 minutes.

3. Remove excess decontaminant from the tray by wiping with a sponge or cloth soaked in a decontaminant. For Class II cabinets, drain the tray into the cabinet base, lift out tray and removable exhaust grille work, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a decontaminant. Then replace in position and drain decontaminant from cabinet base into appropriate container and autoclave according to standard procedures. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.

B. Biohazard Spill Outside a Biological Safety Cabinet

1. Hold your breath, leave the room immediately, and close the door.

2. Warn others not to enter the contaminated area.

3. Remove and put into a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. Wait 30 minutes to allow dissipation of aerosols created by the spill.

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. For a high risk agent, a jumpsuit with tight-fitting wrists and use of a respirator should be considered).

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow an adequate contact time.

8. Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

9. The dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with non autoclavable plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

C. Radioactive Biohazard Spill Outside a Biological Safety Cabinet

In the event that a biohazardous spill also involves a radiation hazard, the clean-up procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances must have the services of a designated radiation protection officer available for consultation.

The following procedure indicates suggested variations from the biohazard spill procedure (above) that should be considered when a radioactive biohazard spill occurs outside a Biological Safety Cabinet.*

1. Holding your breath, leave the room immediately and close the door.

2. Warn others not to enter the contaminated area.

3. Remove and put in a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. Wait thirty minutes to allow dissipation of aerosols created by the spill.

*Before clean-up procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the relative degree of risk.

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting sleeves and a respirator should be considered).

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow adequate disinfectant contact time.

8. *In most cases, the spill will involve ^{14}C or ^3H , which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the clean-up operation is begun.

If the radiation protection officer approves, the biohazard-handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

*If the radiation protection officer determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, sufficient decontaminant solution to immerse the contents should be added to the waste container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive

waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided.

9. If autoclaving has been approved, the dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

*A final radioactive survey should be made of the spill area, dust pan, and squeegee with a Geiger counter, or a smear should be taken and counted in a liquid scintillation counter.

IX. A SECONDARY RESERVOIR AND FILTRATION APPARATUS FOR VACUUM SYSTEMS

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into collection vessels or reservoirs is a common procedure in many laboratories. To prevent the accidental contamination by aerosols or fluids of house vacuum systems or laboratory pumps, some investigators have installed side arm flasks containing cotton, sulfuric acid or decontaminant between the reservoir and the vacuum line. Cotton is not completely effective as a filtering agent, sulfuric acid will corrode pipes, and decontaminants may lose their inactivating ability upon standing. The introduction of a cartridge-type filter that is moisture resistant and has a rated capacity to remove particles 350 nm (0.35u) or larger in size provides an effective barrier to virus aerosols.

The secondary reservoir and filtration apparatus can be assembled from readily available units as shown in Figure 1. A length of plastic tubing $\frac{1}{4}$ inch I.D. $\times \frac{1}{16}$ inch wall is attached at one end of the reservoir and at the other end to the lower arm of a filtration and media storage flask. These flasks vary in capacity from 250 to 4000 ml, the choice of flask depending on available space and amount of fluid that could be accidentally aspirated. A second tube of the same dimensions is attached from the upper arm of the flask to the inlet port of the disposable filter assembly. The third tube is attached from the filter assembly to a vacuum source. The tubes are securely held to the filter by fittings supplied with the filter and the other tubing connections can be secured by worm drive hose clamps.

Ideally the flask should be placed higher than the reservoir of collection vessel. If fluid is accidentally drawn into the flask, the liquid can drain back into the reservoir by gravity if the connection at the vacuum line is broken. This prevents the loss of fluid which the investigator needs to retain.

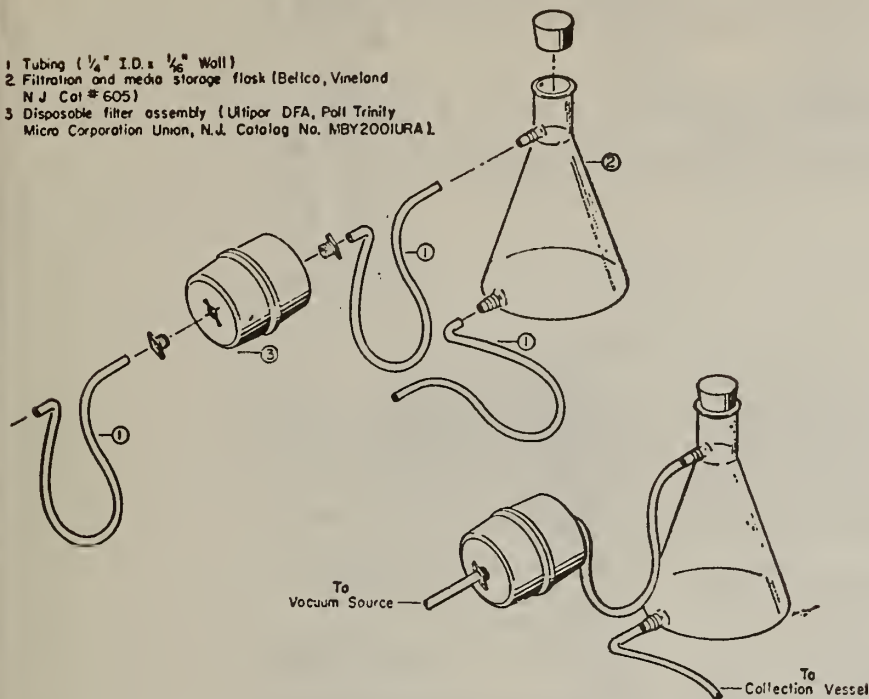
Should the flask be used only for the recovery and storage of waste fluids, then the addition of a few grams of Dow Corning Antifoam A to the flask will reduce violent foaming of fluids aspirated into it. Such fluids can be decontaminated by introducing into the reservoir a final 5% concentration of an iodophor or other appropriate decontaminant, holding for 30 minutes and draining as above.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.

*Changes in procedures have been starred and underlined.

Figure 1
A SECONDARY RESERVOIR AND FILTRATION APPARATUS

- 1 Tubing ($\frac{1}{4}$ " I.D. x $\frac{1}{8}$ " Wall)
- 2 Filtration and media storage flask (Bellco, Vineland N.J. Cat #605)
- 3 Disposable filter assembly (Ultipor DFA, Pall Trinity Micro Corporation Union, N.J. Catalog No. MBY2001URA)



X. PACKAGING AND SHIPPING

A. Introduction

Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all DNA recombinant materials will be packaged and shipped in containers that meet the requirements of these regulations and carrier tariffs. In addition when any portion of the recombinant DNA material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 (which is included at the end of this section, page D-85) the labeling requirements in these regulations and carrier tariffs shall apply.

B. Packaging of Recombinant DNA Materials

1. Volume less than 50 ml.

Material shall be placed in a securely closed, watertight container [primary container (test tube, vial, etc.)] which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of

primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s).

Descriptions of this packaging method are given in Table III.

2. Volumes of 50 ml. or Greater.

Material shall be placed in a securely closed, watertight container (primary container) which shall be enclosed in a second, durable watertight container (secondary container). Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. A shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary con-

tainer and the outer shipping container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of materials which may be enclosed within a single outer shipping container should not exceed 4,000 ml.)

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages which comply with the regulations of the Department of Transportation (DOT) are given in Table IV.

C. Labeling of Packages Containing Recombinant DNA Materials

1. Materials which do not contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. Place only the address label on the outer shipping container. DO NOT USE THE LABEL FOR ETIOLOGIC AGENTS/BIOMEDICAL MATERIAL.

2. Materials which contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in paragraph (c) (4) of 42 CFR 72.25.

3. Materials which contain any portion of a plant pest (plant pathogens) which are so defined by the Department of Agriculture (USDA).

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the shipping labels furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents shall be affixed to the outer shipping container.

D. Additional Shipping Requirements and Limitations for Recombinant DNA Materials

1. Domestic Transportation.

Civil Aeronautics Board Rule No. 82 (Air Transport Association Restricted Articles Tariff 6-D) requires that a Shipper's Certificate, depicted below, be completed and affixed to all shipments which bear the ETIOLOGIC AGENT/BIOMEDICAL MATERIALS label required under the provisions of the Interstate Quarantine regulations [42 CFR Section 72.25(c)]. The Certificate must be completed in duplicate and affixed to the outer shipping container.

This is to certify that the contents of this consignment are properly classified, described by proper shipping name and are packed, marked and labelled and are in proper condition for carriage by air according to all applicable carrier and government regulations. (For international shipments add "and to the IATA Restricted Articles Regulations".) This consignment is within the limitations prescribed for: PASSENGER AIRCRAFT/CARGO ONLY (cross out nonapplicable).

Number of Packages	Specify Each Article Separately (Proper Shipping Name)	Classification	Net Quantity per Package
	ETIOLOGIC AGENT, n.o.s.	ETIO. AG.	

Shipper:

Date: _____

(Signature of Shipper) _____

Shipments of recombinant DNA Materials exceeding 50 ml in volume and containing any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25 are restricted, by DOT regulations, to transport by cargo only aircraft. When the volume of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on the Shipper's Certificate by crossing out "Passenger Aircraft".

When dry ice is used as a refrigerant an "ORA-Group A-DRY ICE LABEL" should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

2. International Transportation.

In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of recombinant DNA materials in which any portion of

the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 must have one or more of the following documents—depending on the country of destination:

(1) Parcel Post Customs Declaration (PS 2966) tag.

(2) Parcel Post Customs Declaration (PS 2966-A) label.

(3) International Parcel Post—Instructions Given by Sender (POD 2922) label.

(4) Dispatch note (POD 2972) tag.

(5) "Violet Label".

(6) Shipper's Certificate specified in the current International Air Transport Association Tariff.

Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

TABLE III.—Description of packages for material in volume less than 50 ml.

Volume (milliliter)	Primary container	Packing	Secondary container	Packing	Outer shipping container
16 maximum	Sealed vial(s) or small glass test tube, screw cap or stopper, taped. ¹	Nonparticulate absorbent material at top, bottom, and sides that will completely absorb contents of the primary container(s).	Metal can 1-in diameter by 7-in outside dimensions metal screw cap.	None required	Fiberbody; metal screw cap top and bottom; 1½-in diameter by 7-to 7½-in outside dimensions.
50 or less	One 20 by 150 mm test tube, taped stopper or multiple small vials. ²	do	Metal can 2½-in diameter by 6½-in high outside dimensions screw cap.	do	Fiberbody; metal screw cap top and bottom; 3¼-in diameter by 7-to 7½-in outside dimensions
Do	Plastic screw cap bottle or Pyrex glass with skirt rubber stopper. ²	do	do	do	Do.
Do	Multiple watertight vials or tubes, taped stoppers. ²	do	1 or more friction-seal tin cans 306 by 400 or larger. ³	None required, but with the 306 by 400 cans or larger cans use sufficient non-particulate shock-absorbent material to prevent rattling.	Fiberboard box

¹ If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the secured (original) outer shipping container. A leak-proof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

² The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription

bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5- or 6-mil polyvinyl tubing heat sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

³ 610 by 708 and 804 by 908 are trade designations for outside dimensions of 610, in diameter by 7½-in height, and 814-in by 9½-in.

TABLE IV.—Description of packages for material in volumes of 50 ml or greater

Volume (milliliters)	Primary container	Packing	Secondary container	Packing			Outer shipping container	
				With refrigerant	Without refrigerant	With refrigerant	With refrigerant	Without refrigerant
51 to 100.....	Plastic or Pyrex glass screw cap bottle, rubber or skirt rubber stopper, taped. ¹	Nonparticulate absorbent material at top, bottom, and sides that will completely absorb contents of the primary container(s).	Consists of metal container and outer container specified in table III.	Styrofoam box shock-absorbent insulation.	Shock absorbent material, in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.	Fiberboard box closely fitting the styrofoam box, taped shut.	Corrugated fiberboard or cardboard box, taped shut.	
100 maximum....	1 100-ml plastic screw cap narrow neck bottle or Pyrex glass, taped. ¹	do.....	No. 3 crimp seal tin can 404 by 700 or a 1-gal friction seal tin can, 610 by 708, top soldered or clipped at 4 points. ¹	do.....	do.....	do.....	V3C cardboard box PS3 type, 9 ¹ / ₂ in by 9 ¹ / ₂ in by 11 ¹ / ₄ in high outside dimensions, taped shut with 3 in type PS3 tape. Do.	
200 maximum....	2 100-ml plastic screw cap bottles or Pyrex glass, taped. ¹	do.....	do. ¹	do.....	do.....	do.....	do.....	
250 maximum....	1 250-ml plastic narrow mouth screw cap bottle or Pyrex glass skirted rubber stopper, taped. ¹	do.....	do. ¹	do.....	do.....	do.....	Do.	
500 maximum....	2 250-ml plastic screw cap bottles or Pyrex glass bottles, taped. ¹	do.....	2-gal friction-seal tin can, 804 by 908, top soldered or clipped at 4 points. ¹	do.....	do.....	do.....	V3C cardboard box 12 ¹ / ₄ in by 12 ¹ / ₄ in by 10 ¹ / ₂ in high outside dimensions, taped shut with 3-in wide PS3 tape.	
Do.....	500-ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500-ml plastic bottle, narrow or wide mouth, screw cap, taped. ¹	do.....	No. 12 crimp seal tin can 603 by 810 2-gal friction seal tin can, 804 by 908, top soldered or clipped at 4 points. ¹	do.....	do.....	do.....	V3C cardboard box 12 ¹ / ₄ in by 12 ¹ / ₄ in by 10 ¹ / ₂ in high outside dimensions, taped shut with 3 in wide PS3 tape. For the No. 12 can a cardboard box is OK taped shut.	

¹ The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

² 610 by 708 and 804 by 908 are trade designations for outside dimensions of 6¹/₈-in diameter by 7³/₈ in height, and 8¹/₈ in by 9¹/₈ in.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; PUBLIC HEALTH SERVICE; CENTER FOR DISEASE CONTROL; ATLANTA, GEORGIA 30333; TELEPHONE: (404) 633-3311, EXT. 3883

TITLE 42—PUBLIC HEALTH; CHAPTER I—PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; SUBCHAPTER F—QUARANTINE, INSPECTION, LICENSING; PART 72—INTERSTATE QUARANTINE; SUBPART C—SHIPMENT OF CERTAIN THINGS

Section 72.25 of Part 72, Title 42, Code of Federal Regulations, is amended to read as follows:

§ 72.25 *Etiologic agents.*

(a) *Definitions.* As used in this section:

(1) An "etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease.

(2) "diagnostic specimen" means any human or animal material including, but not limited to, excreta, secretions, blood and its components, tissue, and tissue fluids being shipped for purposes of diagnosis.

(3) A "biological product" means a biological product prepared and manufactured in accordance with the provisions of 9 CFR Part 10, Licensed Veterinary Biological Products, 42 CFR Part 73, Licensed Human Biological Products, 21 CFR 130.3, *New drugs for investigational use in humans*, 9 CFR Part 103, Biological Products for Experimental Treatment of Animals, or 21 CFR 130.3(a), *New drugs for investigational use in animals*, and which, in accordance with such provisions may be shipped in interstate traffic.

(b) *Transportation; etiologic agent minimum packaging requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, including but not limited to, diagnostic specimens and biological products, containing, or reasonably believed by such person to contain an etiologic agent unless such material is packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

(c) *Transportation; etiologic agents subject to additional requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, other than diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, one or more of the following etiologic agents unless such material is packaged in accordance with the requirements specified in paragraph (b) of this section, and unless, in addition, such material is packaged and shipped in accordance with the requirements specified in subparagraphs (1)–(6) of this paragraph:

¹The requirements of this section are in addition to and not in lieu of any other packaging or other requirements for the transportation of etiologic agents in interstate traffic prescribed by the Department of Transportation and other agencies of the Federal Government.

BACTERIAL AGENTS

Actinobacillus—all species.
Arizona hinshawii—all serotypes.
Bacillus anthracis.
Bartonella—all species.
Bordetella—all species.
Borrelia recurrentis, *B. vincenti*.
Brucella—all species.
Clostridium botulinum, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. tetani*.
Corynebacterium diphtheriae C, equi, C, haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale.
Diplococcus (Streptococcus) pneumoniae.
Erysipelothrix insidiosus.
Escherichia coli, all enteropathogenic serotypes.
Francisella (Pasteurella) tularensis.
Haemophilus ducreyi, *H. influenzae*.
Herellea vagincola.
Klebsiella—all species and all serotypes.
Leptospira interrogans—all serotypes.
Listeria—all species.
Mima polymorpha.
Moraxella—all species.
Mycobacterium—all species.
Mycoplasma—all species.
Neisseria gonorrhoeae, *N. meningitidis*.
Pasteurella—all species.
Pseudomonas pseudomallei.
Salmonella—all species and all serotypes.
Shigella—all species and all serotypes.
Sphaerophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema carereuni, *T. pallidum*, and *T. pertenue*.
Vibrio fetus, *V. comma*, including biotype El Tor, and *V. parahemolyticus*.
Yersenia (Pasteurella) pestis.

FUNGAL AGENTS

Actinomycetes (including *Nocardia* species, *Actinomyces* species and *Arachnia propionica*).
Blastomyces dermatitidis.
Coccidioides immitis.
Cryptococcus neoformans.
Histoplasma capsulatum.
Paracoccidioides brasiliensis.

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

Adenoviruses—human—all types.
Arboviruses.
Coxiella burnetii.
Coxsackie A and B viruses—all types.
Cytomegaloviruses.
Dengue virus.
Echoviruses—all types.
Encephalomyocarditis virus.
Hemorrhagic fever agents, including *Crimean hemorrhagic fever (Congo)*, *Junin*, and *Machupo* viruses, and others as yet undefined.
Hepatitis-associated antigen.
Herpesvirus—all members.
Infectious bronchitis-like virus.
Influenza viruses—all types.
Lassa virus.
Lymphocytic choriomeningitis virus.
Marburg virus.
Measles virus.

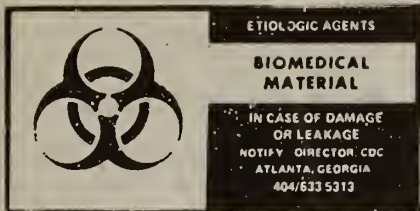
Mumps virus.
Parainfluenza viruses—all types.
Poliomyelitis viruses—all types.
Poxviruses—all members.
Psittacosis-Ornithosis-Trachoma-Lymphogranuloma group of agents.
Rabies virus—all strains.
Reoviruses—all types.
Respiratory syncytial virus.
Rhinoviruses—all types.
Rickettsia—all species.
Rubella virus.
Simian viruses—all types.
Tick-borne encephalitis virus complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses.
Vaccinia virus.
Varicella virus.
Variola major and *Variola minor* viruses.
Vesicular stomatitis virus.
Yellow fever virus.

(1) *Volume less than 50 ml.* Material shall be placed in a securely closed, watertight container (primary container (test tube, vial, etc.)) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

(2) *Volume 50 ml. or greater.* Packaging of material in volumes of 50 ml. or more shall include, in addition, a shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers at the top, bottom, and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single, secondary container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of etiologic agent which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.)

(3) *Dry ice.* If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be so placed that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

(4) *Labels.* The label for Etiologic Agents/ Biomedical Material, except for size and color, must be shown:



(i) The color of material on which the label is printed must be white and the symbol and printing in red.

(ii) The label must be a rectangle measuring 51 mm. (2 inches) high by 102.5 mm. (4 inches) long.

(iii) The red symbol measuring 38 mm. (1½ inches) in diameter must be centered in a white square measuring 51 mm. (2 inches) on each side.

(iv) Type size of the letters of label shall be as follows:

ETIOLOGIC AGENT-----	10 pt. rev.
BIOMEDICAL MATERIAL-----	14 pt.
IN CASE OF DAMAGE OR	
LEAKAGE-----	10 pt. rev.
NOTIFY DIRECTOR CDC AT-	
LANTA, GA-----	8 pt. rev.
404 633 5313-----	10 pt. rev.

(5) *Damaged packages.* Carriers shall promptly, upon discovery of damage to the package that indicates damage to the primary container, isolate the package and notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313), and the sender.

(6) *Registered mail or equivalent system.* Transportation of the following etiologic agents shall be by registered mail or an equivalent system which requires or provides for sending notification to the shipper immediately upon delivery:

Actinobacillus mallei.
Coccidioides immitis.
Francisella (Pasteurella) tularensis.
Hemorrhagic fever agents, including, but not limited to, Crimean hemorrhagic fever (Congo), Junin, Machupo viruses.
Herpesvirus simiae (B virus).
Histoplasma capsulatum.
Lassa virus.
Marburg virus.
Pseudomonas pseudomallei.
Tick-borne encephalitis virus complex. Including, but not limited to, Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses, Variola minor and Variola major.
Yersenia (Pasteurella) pestis.

(d) *Notice of delivery; failure to receive.* When notice of delivery of agents containing, or suspected of containing, etiologic agents listed in paragraph (c) (6) of this section is not received by the sender within 5 days following anticipated delivery of the package, the shipper shall notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313).

(e) *Requirements; variations.* The Administrator may approve variations from the requirements of this section if, upon review and evaluation, he finds that such variations provide protection at least equivalent to that provided by compliance with the requirements specified in this section and makes such findings a matter of official record.

(Sec. 361, 58 Stat. 703; 42 U.S.C. 264)

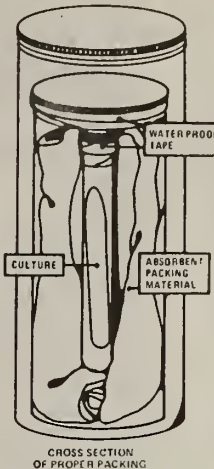
[FR Doc.72-9887 Filed 6-29-72;8:45 am]

Effective July 30, 1972



PACKAGING AND LABELING OF ETIOLOGIC AGENTS

FIGURE 2



CROSS SECTION OF PROPER PACKING

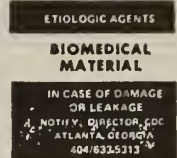
The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph (C) (1) of the cited regulation. Figure 3 illustrates the color and size of the label, described in subparagraph (C) (4) of the regulations, which shall be affixed to all shipments of etiologic agents.

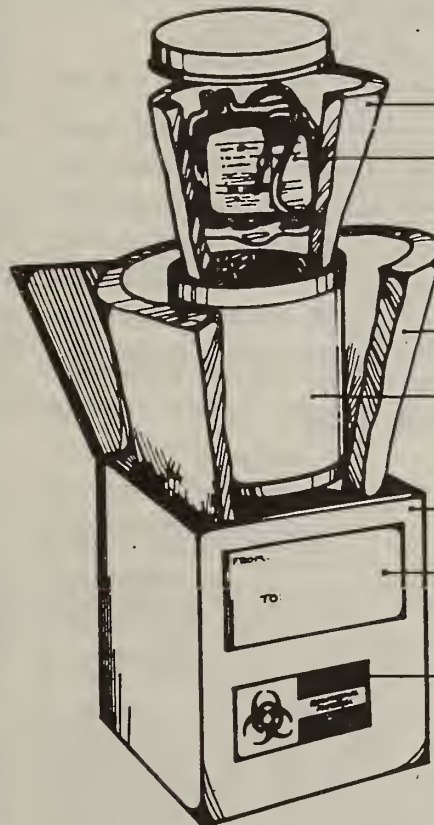
For further information on any provision of this regulation contact:

Center for Disease Control
 Attn: Biohazards Control Office
 1600 Clifton Road
 Atlanta, Georgia 30333
 Telephone: 404 633 3311

FIGURE 3



PACKAGING AND LABELING OF ETIOLOGIC AGENTS



ABSORBENT PACKING MATERIAL

PRIMARY CONTAINER (Bottle, blood bag, etc.)

*NOTE: Single primary containers may not exceed 500 ml. of material. Two or more primary containers whose combined volumes do not exceed 500 ml. may be enclosed in a single, secondary container. The maximum volume of etiologic agent which may be enclosed in a single outer shipping container shall not exceed 4000 ml.

SHOCK ABSORBENT MATERIAL

SECONDARY CONTAINER (Gasketed screwcap with waterproof tape or hermetically sealed can)

OUTER SHIPPING CONTAINER

MAILING LABEL

ETIOLOGIC AGENT LABEL

The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972, to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic. The illustration shows acceptable packaging and labeling of etiologic agents in accordance with subparagraphs (c) (2) and (4) of the cited regulation.

For further information on any provision of this regulation contact:

Center for Disease Control
 Attn: Biohazards Control Office
 1600 Clifton Road
 Atlanta, Georgia 30333

Telephone: 404-633-3311

XI. TRAINING AIDS, MATERIALS AND COURSES

A. Slide-Tape Cassettes

1. Assessment of Risk in the Cancer Virus Laboratory (#10).
2. Effective Use of The Laminar Flow Biological Safety Cabinet (#10).
3. Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets (#10).
4. Certification of Class II (Laminar Flow) Biological Safety Cabinets (#13).
5. Hazard Control in the Animal Laboratory (#10).
6. Basic Principles of Contamination Control (In preparation).
7. Selection of a Biological Safety Cabinet (In preparation). These slide tape cassettes are available for purchase from the National Audiovisual Center. The price for each is given above after the title. Send your order prepaid with a check or money order made payable to National Archives Trust Fund and mail to: Sales Branch, National Audiovisual Center (GSA), Washington, D.C. 20409.

8. Research Laboratory Safety. This slide tape cassette, stock number 17679, is available for \$75 from the National Safety Council, 425 North Michigan Avenue, Chicago, Illinois 60611.

B. Film's

1. Air Sampling for Microbiological Particulates (M-926).
2. Handling the Laboratory Guinea Pig (T2618-X).
3. Handling the Laboratory Mouse (T2617-X).
4. Infectious Hazards of Bacteriological Techniques (M-382).
5. Laboratory Design for Microbiological Safety (M-1091).
6. Plastic Isolators: New Tools for Medical Research (M-599).
7. Safe Handling of Laboratory Animals (M-455).
8. Surface Sampling for Microorganisms (Rodac Method) (M-924).
9. Surface Sampling for Microorganisms (Swab Method) (M-925).

These films are available on loan without charge from: Media Resources Branch, National Medical Audiovisual Center (Annex), Station K, Atlanta, Georgia 30324.

The same films (except 2 and 3) can be rented or bought from: National Audiovisual Center (GSA) (Rental Branch) = (Sales Branch), Washington, D.C. 20409.

C. Courses

1. Biohazard and Injury Control in the Biomedical Laboratory. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries to Dr. Donald Vesley, University of Minnesota, School of Public Health, 1325 Mayo Memorial Building, Minneapolis, Minnesota 55455. June 22-24, 1976, Los Angeles, CA; October 26-28, 1976, Boston, MA; December 7-9, 1976, Bethesda, MD.
2. Biohazard Containment and Control for Recombinant DNA Molecules. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries as above. September 8-9, 1976, Stanford, CA; September 21-11, 1976; Cold Spring Harbor, NY.
3. Safety in Laboratory. Presented by National Institute of Occupational Safety and Health, Division of Training and Manpower Development, by special arrangement. Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226.
4. Laboratory Safety Management. Presented by the Laboratory and Training Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia. September 14-16, 1976, September 13-15, 1977.

XII. OUTLINE OF A SAFETY AND OPERATION MANUAL FOR A P4 FACILITY

A. Purpose

B. Policy

C. Responsibility and Authority

1. Management.
2. Supervisor.
3. Each Employee.
4. Facility Safety Officer.
5. Biohazard Safety Committee.

D. Facility Assignment Procedures

E. Reporting of Major and Minor Accidents and Injuries, Exposure to Toxic or Infectious Materials, Unsafe Conditions and Property Damages, and Rendering First-Aid

F. General Laboratory Safety

1. Fire.
2. Equipment.
3. Physical.
4. Chemical.
5. Radiological.

G. Safety Procedures Associated with Biohazard Activities of the Laboratory

1. Personnel Practices.
2. Operational Practices.

H. Medical Surveillance

I. Facility Operations

1. Personnel Access Procedures.
2. Access Procedures for Equipment Materials and Supplies.
3. Maintenance and Support.
4. Zone Classification.
5. Facility Monitoring Procedures.
6. Housekeeping.

J. Others

1. Packaging and Shipment of Biohazardous Materials.
2. Emergency Procedures.
3. Insect and Rodent Control.
4. Orientation and Training.

Appendix D was prepared by a Working Group Consisting of:

- W. Emmett Barkley (Chairman), National Cancer Institute, NIH.
 Manuel S. Barbeito, National Cancer Institute, NIH.
 Everett Hanel, Jr., Frederick Cancer Research Center.
 George S. Michaelsen, School of Public Health, University of Minnesota.
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 John Richardson, Center for Disease Control.
 James F. Sullivan, National Animal Disease Laboratories.
 Arnold G. Wedum, Frederick Cancer Research Center.

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[FR Doc.76-19151 Filed 7-6-76;8:45 am]

May 28, 1975

Dr. Robert G. Martin
Laboratory of Molecular Biology
National Institute of Arthritis, Metabolism
and Digestive Diseases
Building 2, Room 208
Bethesda, Maryland 20014

Dear Dr. Martin:

I am very pleased to transmit to you the Report of the Organizing Committee of the Asilomar Conference on Recombinant DNA Molecules, which was supported by National Institutes of Health Contract N01-OD-5-2103.

The Summary of the Report, Appendix I, is to be published by Nature, Science, and the Proceedings of the National Academy of Sciences.

If you have any questions about the Report, please do not hesitate to call me.

Sincerely,

Artemis P. Simopoulos, M.D.
Executive Secretary

APS:JP

Enclosure

Report of the Organizing Committee of the Asilomar Conference on Recombinant DNA Molecules

In its report to the Assembly of Life Sciences of the National Research Council (Science 185:303 (1974)), the Committee on Recombinant DNA Molecules urged that an international meeting of involved scientists from throughout the world be convened to review scientific progress in this area and to discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules. The Asilomar Conference on Recombinant DNA Molecules met during February 24-27, 1975, at the Asilomar Conference Center in Pacific Grove, California. This report, drafted by the Organizing Committee* (Paul Berg, Chairman, David Baltimore, Sydney Brenner, Richard Roblin and Maxine Singer), summarizes the organization, proceedings and recommendations of the Conference.

The Conference was sponsored by the U.S. National Academy of Sciences with financial support from the National Cancer Institute of the National Institutes of Health and the National Science Foundation. Besides providing the administrative and travel costs needed to organize the meeting, these funds paid the travel and living expenses for many of the U.S. and foreign participants.

To ensure both the greatest opportunity for effective discussions of the relevant scientific issues and the most frank and searching analysis of the potential risks, attendance at the Conference was limited. Individuals receiving invitations from the Organizing Committee were a) chosen by the chairmen responsible for the scientific and discussion sessions, b) presently engaged in or contemplating researches with recombinant DNA molecules and c) experts who could provide special information and insight to the question of assessing and dealing with the potential hazards of such work. Though official representation from U.S. as well as foreign scientific and governmental agencies was not sought, many of these organizations were informed as to those that had been invited to participate in the Conference.

Of the 155 participants, 83 were from U.S. research, governmental and industrial institutions, 51 were from comparable organizations outside the U.S. and 21 represented the lay and scientific news media. (Appendix A provides the names and affiliations of participants.)

Though there would have been some advantages to publishing the Proceedings of the Conference, the Organizing Committee chose not to pursue this course. Instead, the presentations and discussions were recorded on audio tape; these recordings, therefore, provide a verbatim record of the transactions. Inasmuch as the Conference participants were assured that the recordings would be used for archival purposes only, we propose that the seven reels of audio tape and their diaries (Appendix B) should be retained by the Academy for future reference and that their copying or distribution be proscribed.

The Conference program (Appendix C) focused on five subject areas: Ecology of plasmids and enteric bacteria; molecular biology of prokaryote plasmids and their use for molecular cloning; synthetic recombinants involving animal virus DNAs; synthetic recombinants involving eukaryote DNAs; and ethical and legal concerns arising from work on synthetic recombinant DNAs. The responsibility for organizing the formal presentations and the panel-led discussions in each subject area was given to a group of four to six individuals organized by a program chairman. (Subsequently the working groups assigned the first two subject areas merged and functioned as a single group for purposes of program preparation and presentation.) Each group met once or twice prior to the Conference to select speakers and discussors, to organize their formal scientific presentations and to draft working papers for distribution and use at the Conference (Appendices D, E, F). Our intent was to permit the experts in each area to select and organize the presentations most relevant to the scientific and biohazard issue. Moreover, these groups were encouraged to prepare written statements containing propositions and recommendations for assessing and dealing with the real and possible biohazards. These planning groups also met with other participants during the Conference to explore the implications and consequences of

their recommendations. Several unscheduled "workshops" were also held during "free" times to design and plan construction of safer cloning vehicles (plasmids and phages) and safer bacterial hosts (see Appendix G). The nearly unanimous acceptance of the recommendations emerging from the Conference indicates that these unscheduled events were useful and germinal.

The task of summarizing the views and conclusions developed at the Conference was accepted by the Organizing Committee. A statement, entitled "Provisional Statement of the Conference Proceedings" (Appendix H) was drafted at the end of the third day of the Conference and served as the working document for the discussion during the final session of the meeting. As expected, the conclusions and recommendations offered in the document elicited contrary views, particularly as to the assessment of the risks and how to deal with them. Each section of the provisional statement was debated, suggestions for deletions, additions, and modifications in the wording were considered and in the end the Conference participants voted by hands for adoption or rejection of the various amended propositions. Left unchallenged, and supported by the overwhelming majority of the participants, was the recommendation that most of the research with synthetic recombinant DNAs could proceed provided that the experiments were performed under conditions which ensure a minimum of risk to the experimenters and the public at large. Moreover, there was virtually unanimous acceptance of the principle that adequate containment, both physical and biological, must be considered an essential feature of each particular experimental protocol. At the meeting's end it was agreed that the Organizing Committee would redraft the statement so that it would reflect the discussions and positions adopted by the balloting at the Conference as well as views offered by written submissions following the Conference.

The agreed upon principles, together with more specific recommendations and guidelines, are included in a revised statement entitled "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (Appendix I). Inasmuch as section 5 of the Provisional

Statement of the Conference Proceedings (Appendix H) was not discussed by the participants and because the proposal is appropriate primarily to United States institutions, it has been omitted from the revised summary statement. But we believe a proposal as to how compliance with any promulgated containment guidelines could be monitored would be useful. Accordingly, we have included an outline for a plausible model of how such a monitoring process could function (Appendix J).

We recommend that the statement designated Appendix I be published as quickly as possible in appropriate scientific journals, e.g., Science, Nature and the Proceedings of the National Academy of Sciences, and that the statement also be distributed to the Conference participants as soon as its contents have been approved by the Academy's review process.

*Though Dr. Niels Jerne was appointed a member of the Organizing Committee, he did not participate in the pre-Conference activities and was unable to attend the Conference; consequently he did not share in writing this report.

NATIONAL ACADEMY OF SCIENCES
International Conference on Recombinant DNA Molecules
February 24-27, 1975 Asilomar Conference Center

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Provisional Statement of the Conference Proceedings

1. This meeting was organized to review scientific progress in the area of recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. We have heard about the enormous scientific progress already achieved in this field, and have seen glimpses of the remarkable potential of these methods to accelerate the rate at which we may gain understanding of the fundamental processes occurring in eukaryotic cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. While there has as yet been no practical application of the new techniques, there is every reason to believe that it will have significant impact in the future.

The participants at the meeting agreed that the pause in research, called for in the July 1974 committee letter, ought not to be left unresolved. They considered whether there were ways in which the scientific work could be undertaken with minimal risks to the workers in laboratories and to society at large. It was emphasized that, in the longer term, even more difficult problems may arise in the probably large scale applications of this work in industry, medicine and agriculture. Even in the currently more limited area of the conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. The new techniques combining genetic information from very different organisms place us in an area of biology with many unknowns. It is this ignorance that has compelled us to conclude that it would be wise to exercise the utmost caution. Nevertheless, the work should proceed but with appropriate safeguards. Although future experience may dispel many fears, standards of protection should be set high at the beginning and each escalation, however small, should be carefully assessed.

2. Though our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free of any risk. Reasonable principles for dealing with these risks are to adopt containment as a part of the experimental strategy and that the effectiveness of the containment should match the risk. Whatever scale of risks is ultimately devised, we shall need a commensurate scale of containment. Consequently we must seek means for estimating the risks, perhaps subjectively at first but objectively as we acquire additional knowledge, and then to match that risk to the appropriate degree of containment. Experiments requiring large scale operations would seem to be riskier than the equivalent experiment done on a small scale, and, therefore, require more stringent containment procedures. Improvements in the methodology. e.g., a "disarmed" vector or host cell, could permit a reduction in the containment requirement. Quite possibly the ways in which potential biohazards and different levels of containment are matched may vary from country to country; also, the ways could vary from time to time as the containment technology is improved. Thus, it is essential that there be a continued reassessment of the balancing of risk against level of containment.

Multiple factors define each level in the scale of containment. The most important factors, because they contribute most significantly to limiting the spread of the recombined plasmids, are biological barriers. These barriers are of two types; fastidious bacterial hosts unable to survive in natural environments and non-transmissible vectors designed to grow only in specified hosts. A second factor is adherence to good microbiological practices which, to a large measure, can limit the escape of organisms from the experimental situation. Physical containment, exemplified by the use of suitable hoods, or, where applicable, contained laboratories is an additional factor. Further, education and training of all personnel involved in the experiments is essential to the effectiveness of all of the above.

3. Specific Recommendations

These recommendations reflect the principle that a serious evaluation of biohazard potential and the adoption of appropriate biological and physical barriers are integral parts of experiments with recombinant DNA molecules. In this section three levels of containment affording increasing protection are defined. Then, various types of experiments are evaluated for potential biohazards and matched with appropriate containment levels.

A. Types of Containment

The types of containment are an adaptation of the "NCI Safety Standards".

1) Low-This type of containment involves basic good medical microbiological techniques. The essential factors are wearing lab coats, using mechanical pipettes, no eating in the lab, and that sonication and other procedures which generate large aerosols should be done in biological safety cabinets.

While existing vectors can be used for this level of containment, as safer vectors become available, their use is recommended.

2) Moderate-Both physical and biological containment enter into this containment system.

The physical containment for moderate risk agents was designed for handling moderate risk oncogenic viruses. The main features are that transfer operations are to be carried out in biological safety cabinets, gloves are worn in addition to lab coats, vacuum lines are protected by filters, and negative pressure is maintained in limited access laboratories.

The physical containment procedures are recognized to provide significant but incomplete protection against the accidental spread of biological agents. The potential hazard of moderate risk agents is such that a strong measure of biological containment is needed to ensure their safe handling. Therefore, experiments with such agents should only use prokaryotic vectors which have been designed with increased safety in mind. Such vectors are currently being designed and created, and should be available in the near future so that these experiments can proceed.

3) High-This type of containment involves facilities which are isolated from other areas by air locks, clothing changes and shower rooms and which have treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air, liquid and solid wastes. The handling of agents shall be confined to biological safety cabinets and all persons occupying these areas shall wear only laboratory protective clothing and shall shower at each exit from the containment facility. In addition the containment facility shall be maintained under negative air pressure.

Again, only vectors designed for safety should be used.

B. Types of Experiments

The letter published by the Committee on Recombinant DNA Molecules in July 1974 requested that the scientific community join the Committee in deferring two types of experiments. The letter also advised caution regarding a third type of experiment. In the following assessments of potential hazards the three original categories are maintained, but with some redefinition.

1) Prokaryotes - Potential biohazards of experiments involving genetic exchange among prokaryotic organisms can in general be accurately assessed.

Experiments involving organisms that normally exchange genetic information involve no novel biotypes and pose no hazards that cannot be contained by the standard microbiological laboratory techniques appropriate for the handling of these organisms.

Experiments involving the introduction of bacterial genes into species in which they have not been found to occur naturally result in novel biotypes and so pose increased potential biohazards. Such experiments involving genetic determinants affecting pathogenicity for man or other species or antibiotic resistance should be undertaken only under conditions of moderate or high containment; any large scale industrial, commercial, agricultural or other applications should be deferred pending the issuance of appropriate official guidelines by national scientific bodies.

2) Viruses - Experiments employing "low risk" animal viruses as vectors to introduce new genetic material into animal cells can be carried out under moderate physical containment conditions.

Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their introduction into prokaryotic cells should be carried out under moderate containment conditions including improved vectors. Rigorously purified fragments of the demonstrably non-transforming regions of oncogenic viral DNAs or regions of non-oncogenic viruses could be attached to plasmid DNAs and introduced into E. coli under moderate risk conditions with existing vectors.

Experiments involving high risk viruses should be carried out under high containment.

3) Eukaryotic DNA - In the low risk category are most experiments involving the fusion of prokaryotic vectors with DNA from prokaryotes, lower eukaryotes, plants, invertebrates and cold-blooded vertebrates. Moderate risk experiments involve the joining of DNA of warm-blooded vertebrates to prokaryotic vectors or the construction of recombinants between animal vectors and any DNA. Experiments with a high risk include the fusion of eukaryotic or prokaryotic genes to prokaryotic vectors when the resultant organism is likely to express a toxic or a pharmacologically active agent.

4. Implementation

It is clear that in many countries steps are already being taken by national bodies to formulate and establish guidelines. Until such guidelines are established we urge individual scientists to use the present document as a guide. There are in addition some recommendations which need not wait until that time and which the scientific community could implement directly.

A. Development of Vectors and Hosts.

One of the most important and interesting accomplishments of the meeting was the beginning of the design and construction of bacteria and vectors which could radically improve the safety of these procedures by many orders of magnitude. It is certain that in the near future there will become available special variants of λ phage, non-transmissible plasmids and special E. coli which will not only optimize safety but will also bring about considerable technical improvements in the methods themselves. Other bacterial systems, particularly suitably modified strains of B. subtilis, may also be specially useful for particular purposes. There is also the possibility that a suitable vector may be found for simple eukaryotic cells. We think that work which aims to improve the vectors or hosts should be given high priority.

B. Laboratory Procedures

Before any experiments of this kind are initiated, the research staff of the laboratory shall be informed of all of the hazards that might be associated with such experiments. Laboratory workers must also be trained in the containment procedures that are designed to control the hazards. This training must include emergency procedures that are to be performed in the event of an accident. It is also strongly recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically to establish a base for epidemiological analyses.

C. Education

A continuing reassessment of these problems to take into account and developing scientific knowledge is essential. This could be achieved by a series of annual courses, workshops and meetings which would also serve to train individuals in the relevant methods. Consideration should also be given to the establishment of a newsletter for the rapid dissemination of new information pertinent to the effectiveness of biological containment.

5. Proposed Model Containment Review Process

A review process should be established which would be able to determine whether a given laboratory has the appropriate containment facilities for a given type of experiment. As far as possible, the biohazard review process should not lengthen the time required for review of research proposals. The specific form of the review procedure in different countries for different scientific and industrial laboratories must depend on local circumstances. The following proposal is, therefore, presented as a model. The model is designed for universities in the United States but would have to be modified for other situations.

Each university or research institution should have a committee empowered and trained to grade the physical containment facilities of its laboratories (e.g., low, moderate or high according to established guidelines). The local committee would provide the laboratory head with a statement certifying the containment rating of the laboratory (subject to periodic reevaluation).

When an individual applies to an agency for funds to support work on recombinant DNA molecules, the certificate of containment rating would be appended. The group reviewing the grant would then determine whether the certified level of containment matched whatever biohazard might result from the proposed work. The biological barriers incorporated in the experiment, the magnitude of proposed growth of bacteria, the type of DNA to be cloned, etc., would all enter into the decision. If the reviewing group is satisfied, the grant would be processed for scientific merit in the usual fashion. If a question arises concerning the appropriateness of the certified containment level, the NIH Advisory Committee on Recombinant DNA Molecules or some other body would be asked for an opinion or ruling.

This procedure would not guarantee that all experiments would be performed under the required containment conditions but if the investigators have reasonable good will, the system would generate widespread compliance.

6. New Knowledge

This document represents our best assessment of the potential biohazards in the light of current knowledge. To improve this assessment, it will be important to have answers to the following questions.

(1) Are eukaryotic genes or viruses expressed in prokaryotic hosts and, if so, can they modify the biohazard potential of these cells?

(2) Can free DNA molecules infect animals or plants?

(3) Can prokaryote-eukaryote recombinant DNA molecules, either free or encapsulated as phage particles infect animal or plant cells and be expressed there?

(4) Can mammalian cells in culture be genetically transformed by free homologous or heterologous DNA?

(5) Can hybrid animal virus DNA or virus-plasmid hybrids cause tumors in animals?

(6) Can methods be developed to monitor effectively the escape and dissemination of cloning vehicles?

Summary Statement of the Asilomar Conference on Recombinant DNA Molecules

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MAXINE F. SINGER

Reprinted from the *Proceedings of The National Academy of Sciences*
Vol. 72, No. 6, pp. 1981-1984. June, 1975.

Summary Statement of the Asilomar Conference on Recombinant DNA Molecules*

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I. INTRODUCTION AND GENERAL CONCLUSIONS

This meeting was organized to review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. Impressive scientific achievements have already been made in this field and these techniques have a remarkable potential for furthering our understanding of fundamental biochemical processes in pro- and eukaryotic cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future.

Of particular concern to the participants at the meeting was the issue of whether the pause in certain aspects of research in this area, called for by the Committee on Recombinant DNA Molecules of the National Academy of Sciences, U.S.A. in the letter published in July, 1974** should end; and, if so, how the scientific work could be undertaken with minimal risks to workers in laboratories, to the public at large, and to the animal and plant species sharing our ecosystems.

The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the Conference agreed that most of the work on construction of recombinant DNA molecules should proceed provided that appropriate safeguards, principally biological and physical barriers ade-

quate to contain the newly created organisms, are employed. Moreover, the standards of protection should be greater at the beginning and modified as improvements in the methodology occur and assessments of the risks change. Furthermore, it was agreed that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. In the longer term, serious problems may arise in the large scale application of this methodology in industry, medicine, and agriculture. But it was also recognized that future research and experience may show that many of the potential biohazards are less serious and/or less probable than we now suspect.

II. PRINCIPLES GUIDING THE RECOMMENDATIONS AND CONCLUSIONS

Although our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free from any risk. Reasonable principles for dealing with these potential risks are: (i) that containment be made an essential consideration in the experimental design and, (ii) that the effectiveness of the containment should match, as closely as possible, the estimated risk. Consequently, whatever scale of risks is agreed upon, there should be a commensurate scale of containment. Estimating the risks will be difficult and intuitive at first but this will improve as we acquire additional knowledge; at each stage we shall have to match the potential risk with an appropriate level of containment. Experiments requiring large scale operations would seem to be riskier than equivalent experiments done on a small scale and, therefore, require more stringent containment procedures. The use of cloning vehicles or vectors (plasmids, phages) and bacterial hosts with a restricted capacity to multiply outside of the laboratory would reduce the potential biohazard of a particular experiment. Thus, the ways in which potential biohazards and different levels of containment are matched may vary from time to time, particularly as the containment technology is improved. The means for assessing and balancing risks with appropriate levels of containment will need to be reexamined from time to time. Hopefully, through both formal and informal channels of information within and between the nations of the world, the way in which potential biohazards and levels of containment are matched would be consistent.

* Summary statement of the report submitted to the Assembly of Life Sciences of the National Academy of Sciences and approved by its Executive Committee on 20 May 1975.

Requests for reprints should be addressed to: Division of Medical Sciences, Assembly of Life Sciences, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

** Report of Committee on Recombinant DNA Molecules: "Potential Biohazards of Recombinant DNA Molecules," *Proc. Nat. Acad. Sci. USA* 71, 2593-2594, 1974.

Containment of potentially biohazardous agents can be achieved in several ways. The most significant contribution to limiting the spread of the recombinant DNAs is the use of biological barriers. These barriers are of two types: (i) fastidious bacterial hosts unable to survive in natural environments, and (ii) nontransmissible and equally fastidious vectors (plasmids, bacteriophages, or other viruses) able to grow only in specified hosts. Physical containment, exemplified by the use of suitable hoods, or where applicable, limited access or negative pressure laboratories, provides an additional factor of safety. Particularly important is strict adherence to good microbiological practices which, to a large measure can limit the escape of organisms from the experimental situation, and thereby increase the safety of the operation. Consequently, education and training of all personnel involved in the experiments is essential to the effectiveness of all containment measures. In practice, these different means of containment will complement one another and documented substantial improvements in the ability to restrict the growth of bacterial hosts and vectors could permit modifications of the complementary physical containment requirements.

Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the possibility of spreading potentially hazardous agents. Therefore, investigators relying upon "disarmed" hosts and vectors for additional safety must rigorously test the effectiveness of these agents before accepting their validity as biological barriers.

III. RECOMMENDATIONS FOR MATCHING TYPES OF CONTAINMENT WITH TYPES OF EXPERIMENTS

No classification of experiments as to risk and no set of containment procedures can anticipate all situations. Given our present uncertainties about the hazards, the parameters proposed here are broadly conceived and meant to provide provisional guidelines for investigators and agencies concerned with research on recombinant DNAs. However, each investigator bears a responsibility for determining whether, in his particular case, special circumstances warrant a higher level of containment than is suggested here.

A. Types of containment

1. *Minimal Risk.* This type of containment is intended for experiments in which the biohazards may be accurately assessed and are expected to be minimal. Such containment can be achieved by following the operating procedures recommended for clinical microbiological laboratories. Essential features of such facilities are no drinking, eating, or smoking in the laboratory, wearing laboratory coats in the work area, the use of cotton-plugged pipettes or preferably mechanical pipetting devices, and prompt disinfection of contaminated materials.

2. *Low Risk.* This level of containment is appropriate for experiments which generate novel biotypes but where the available information indicates that the recombinant DNA cannot alter appreciably the ecological behavior of the recipient species, increase significantly its pathogenicity, or prevent effective treatment of any resulting infections. The key features of this containment (in addition to the minimal procedures mentioned above) are a prohibition on mouth pipetting, access limited to laboratory personnel, and the

use of biological safety cabinets for procedures likely to produce aerosols (e.g., blending and sonication). Though existing vectors may be used in conjunction with low risk procedures, safer vectors and hosts should be adopted as they become available.

3. *Moderate Risk.* Such containment facilities are intended for experiments in which there is a probability of generating an agent with a significant potential for pathogenicity or ecological disruption. The principle features of this level of containment, in addition to those of the two preceding classes, are that transfer operations should be carried out in biological safety cabinets (e.g., laminar flow hoods), gloves should be worn during the handling of infectious materials, vacuum lines must be protected by filters, and negative pressure should be maintained in the limited access laboratories. Moreover, experiments posing a moderate risk must be done only with vectors and hosts that have an appreciably impaired capacity to multiply outside of the laboratory.

4. *High Risk.* This level of containment is intended for experiments in which the potential for ecological disruption or pathogenicity of the modified organism could be severe and thereby pose a serious biohazard to laboratory personnel or the public. The main features of this type of facility, which was designed to contain highly infectious microbiological agents, are its isolation from other areas by air locks, a negative pressure environment, a requirement for clothing changes and showers for entering personnel, and laboratories fitted with treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air and liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the containment facility. The handling of agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters. High risk containment includes, in addition to the physical and procedural features described above, the use of rigorously tested vectors and hosts whose growth can be confined to the laboratory.

B. Types of experiments

Accurate estimates of the risks associated with different types of experiments are difficult to obtain because of our ignorance of the probability that the anticipated dangers will manifest themselves. Nevertheless, experiments involving the construction and propagation of recombinant DNA molecules using DNAs from (i) prokaryotes, bacteriophages, and other plasmids, (ii) animal viruses, and (iii) eukaryotes have been characterized as minimal, low, moderate, and high risks to guide investigators in their choice of the appropriate containment. These designations should be viewed as interim assignments which will need to be revised upward or downward in the light of future experience.

The recombinant DNA molecules themselves, as distinct from cells carrying them, may be infectious to bacteria or higher organisms. DNA preparations from these experiments, particularly in large quantities, should be chemically inactivated before disposal.

1. *Prokaryotes, Bacteriophages, and Bacterial Plasmids.* Where the construction of recombinant DNA molecules and their propagation involves prokaryotic agents that are known to exchange genetic information naturally, the experiments

can be performed in minimal risk containment facilities. Where such experiments pose a potential hazard, more stringent containment may be warranted.

Experiments involving the creation and propagation of recombinant DNA molecules from DNAs of species that ordinarily do not exchange genetic information, generate novel biotypes. Because such experiments may pose biohazards greater than those associated with the original organisms, they should be performed, at least, in low risk containment facilities. If the experiments involve either pathogenic organisms or genetic determinants that may increase the pathogenicity of the recipient species, or if the transferred DNA can confer upon the recipient organisms new metabolic activities not native to these species and thereby modify its relationship with the environment, then moderate or high risk containment should be used.

Experiments extending the range of resistance of established human pathogens to therapeutically useful antibiotics or disinfectants should be undertaken only under moderate or high risk containment, depending upon the virulence of the organism involved.

2. Animal Viruses. Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their propagation in prokaryotic cells should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and with moderate risk containment facilities. Rigorously purified and characterized segments of non-oncogenic viral genomes or of the demonstrably non-transforming regions of oncogenic viral DNAs can be attached to presently existing vectors and propagated in moderate risk containment facilities; as safer vector-host systems become available such experiments may be performed in low risk facilities.

Experiments designed to introduce or propagate DNA from non-viral or other low risk agents in animal cells should use only low risk animal DNAs as vectors (e.g., viral, mitochondrial) and manipulations should be confined to moderate risk containment facilities.

3. Eukaryotes. The risks associated with joining random fragments of eukaryote DNA to prokaryotic DNA vectors and the propagation of these recombinant DNAs in prokaryotic hosts are the most difficult to assess.

A priori, the DNA from warm-blooded vertebrates is more likely to contain cryptic viral genomes potentially pathogenic for man than is the DNA from other eukaryotes. Consequently, attempts to clone segments of DNA from such animal and particularly primate genomes should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and in a moderate risk containment facility. Until cloned segments of warm-blooded vertebrate DNA are completely characterized, they should continue to be maintained in the most restricted vector-host system in moderate risk containment laboratories; when such cloned segments are characterized, they may be propagated as suggested above for purified segments of virus genomes.

Unless the organism makes a product known to be dangerous (e.g., toxin, virus), recombinant DNAs from cold-blooded vertebrates and all other lower eukaryotes can be constructed and propagated with the safest vector-host system available in low risk containment facilities.

Purified DNA from any source that performs known functions and can be judged to be non-toxic, may be cloned with currently available vectors in low risk containment facilities. (Toxic here includes potentially oncogenic products or substances that might perturb normal metabolism if produced in an animal or plant by a resident microorganism.)

4. Experiments to be Deferred. There are feasible experiments which present such serious dangers that their performance should not be undertaken at this time with the currently available vector-host systems and the presently available containment capability. These include the cloning of recombinant DNAs derived from highly pathogenic organisms (i.e., Class III, IV, and V etiologic agents as classified by the United States Department of Health, Education and Welfare), DNA containing toxin genes, and large scale experiments (more than 10 liters of culture) using recombinant DNAs that are able to make products potentially harmful to man, animals, or plants.

IV. IMPLEMENTATION

In many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard.^{††,‡‡} Until these are established, we urge individual scientists to use the proposals in this document as a guide. In addition, there are some recommendations which could be immediately and directly implemented by the scientific community.

A. Development of safer vectors and hosts

An important and encouraging accomplishment of the meeting was the realization that special bacteria and vectors which have a restricted capacity to multiply outside the laboratory can be constructed genetically, and that the use of these organisms could enhance the safety of recombinant DNA experiments by many orders of magnitude. Experiments along these lines are presently in progress and in the near future, variants of λ bacteriophage, non-transmissible plasmids, and special strains of *Escherichia coli* will become available. All of these vectors could reduce the potential biohazards by very large factors and improve the methodology as well. Other vector-host systems, particularly modified strains of *Bacillus subtilis* and their relevant bacteriophages and plasmids, may also be useful for particular purposes. Quite possibly safe and suitable vectors may be found for eukaryotic hosts such as yeast and readily cultured plant and animal cells. There is likely to be a continuous development in this area and the participants at the meeting agreed that improved vector-host systems which reduce the biohazards of recombinant DNA research will be made freely available to all interested investigators.

B. Laboratory procedures

It is the clear responsibility of the principal investigator to inform the staff of the laboratory of the potential hazards of

^{††} Advisory Board for the Research Councils, "Report of the Working Party on the Experimental Manipulation of the Genetic Composition of Micro-Organisms. Presented to Parliament by the Secretary of State for Education and Science by Command of Her Majesty, January 1975." London: Her Majesty's Stationery Office, 1975, 23pp.

^{‡‡} National Institutes of Health Recombinant DNA Molecule Program Advisory Committee.

such experiments before they are initiated. Free and open discussion is necessary so that each individual participating in the experiment fully understands the nature of the experiment and any risk that might be involved. All workers must be properly trained in the containment procedures that are designed to control the hazard, including emergency actions in the event of a hazard. It is also recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically.

C. Education and reassessment

Research in this area will develop very quickly and the methods will be applied to many different biological problems. At any given time it is impossible to foresee the entire range of all potential experiments and make judgments on them. Therefore, it is essential to undertake a continuing reassessment of the problems in the light of new scientific knowledge. This could be achieved by a series of annual workshops and meetings, some of which should be at the international level. There should also be courses to train individuals in the relevant methods since it is likely that the work will be taken up by laboratories which may not have had extensive experience in this area. High priority should also be given to research that could improve and evaluate the containment effectiveness of new and existing vector-host systems.

V. NEW KNOWLEDGE

This document represents our first assessment of the potential biohazards in the light of current knowledge. However, little is known about the survival of laboratory strains of bacteria and bacteriophages in different ecological niches in the outside

world. Even less is known about whether recombinant DNA molecules will enhance or depress the survival of their vectors and hosts in nature. These questions are fundamental to the testing of any new organism that may be constructed. Research in this area needs to be undertaken and should be given high priority. In general, however, molecular biologists who may construct DNA recombinant molecules do not undertake these experiments and it will be necessary to facilitate collaborative research between them and groups skilled in the study of bacterial infection or ecological microbiology. Work should also be undertaken which would enable us to monitor the escape or dissemination of cloning vehicles and their hosts.

Nothing is known about the potential infectivity in higher organisms of phages or bacteria containing segments of eukaryotic DNA and very little about the infectivity of the DNA molecules themselves. Genetic transformation of bacteria does occur in animals, suggesting that recombinant DNA molecules can retain their biological potency in this environment. There are many questions in this area, the answers to which are essential for our assessment of the biohazards of experiments with recombinant DNA molecules. It will be necessary to ensure that this work will be planned and carried out; and it will be particularly important to have this information before large scale applications of the use of recombinant DNA molecules is attempted.

The work of the committee was assisted by the National Academy of Sciences-National Research Council Staff: Artemis P. Simopoulos (Executive Secretary) and Elena O. Nightingale (Resident Fellow), Division of Medical Sciences, Assembly of Life Sciences, and supported by the National Institutes of Health (Contract NO1-OD-5-2103) and the National Science Foundation (Grant GBMS75-05293).

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APRIL 1976

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NATIONAL INSTITUTES OF HEALTH

PROPOSED GUIDELINES FOR

RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

JANUARY 1976

I. Introduction

The purpose of this report is to recommend safeguards for research on recombinant DNA molecules to the National Institutes of Health and to other institutions that support such research. In this context we define recombinant DNAs as molecules that consist of different segments of DNA which have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome.

The scientific achievements that have already been obtained through the use of recombinant DNAs and the obvious, immediate potentialities of this methodology indicate that we are in the initial phase of a process that promises to revolutionize both biological research and its practical applications. The unique feature of these new methods that makes them so useful is that they provide a means for combining genes from diverse organisms that do not normally exchange genetic information in nature. It is this same characteristic that has caused concern and speculation about the potential biohazards that may result from such novel recombinants (1-3).

This is the first attempt to provide a detailed set of guidelines for use by study sections as well as practicing scientists for evaluating research on recombinant DNA molecules. We cannot hope to anticipate all possible lines of imaginative research that are possible with this powerful new methodology. Nevertheless, the Committee has received a considerable volume of written and verbal contributions from scientists in a variety of disciplines. In many instances the views presented to us were contradictory.

At present, the hazards may be guessed at, speculated about, or voted upon, but they cannot be known absolutely in the absence of firm experimental data--and, unfortunately, the needed data were, more often than not, unavailable. Our problem then has been to construct guidelines that allow the promise of the methodology to be realized while advocating the considerable caution that is demanded by what we and others view as potential hazards.

In designing these guidelines we have adopted the following principles, which are consistent with the general conclusions that were formulated at the International Conference on Recombinant DNA Molecules held at Asilomar Conference Center, Pacific Grove, California, in February 1975 (3):

- (i) There are certain experiments for which the assessed potential hazard is so serious that they should not be attempted at the present time.
- (ii) The remainder can be undertaken at the present time provided that the experiment is justifiable on the basis that new knowledge or benefits to humankind will accrue that cannot readily be obtained by use of conventional methodology and that appropriate safeguards are incorporated into the design and execution of the experiment. In addition to an insistence on the practice of good microbiological techniques, these safeguards consist of providing both physical and biological barriers to the dissemination of the potentially hazardous agents.
- (iii) The level of containment provided by these barriers should match the estimated potential hazard for each of the different classes of recombinants. For projects in a given class, this level should be highest at initiation and modified subsequently only if there is a substantiated change in the

assessed risk or in the applied methodology. (iv) The guidelines will be subjected to periodic review (at least annually) and modified to reflect improvements in our knowledge of the potential biohazards and of the available safeguards.

In constructing these guidelines it has been necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account existing and anticipated special procedures and information that will allow particular experiments to be carried out under different conditions than indicated here without sacrifice of safety. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that study sections give consideration to such procedures which may allow change in the containment levels recommended here. Our recommendations should, therefore, be considered as guidelines in the most literal sense.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and laboratory procedures applicable to organisms carrying recombinant DNAs (4-13). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism--namely, the application of highly specific biological barriers. In fact, natural barriers do exist which either limit the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the guidelines for research on the different kinds of recombinant DNAs (Section III).

A. Standard practices and training - The first principle of containment is a strict adherence to good microbiological practices (4-13). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aseptic techniques and instruction in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

In addition to aseptic experimental techniques, standard practices generally include the following procedures when the experiments involve organisms that may be pathogenic or may undesirably alter the basic ecology:

(i) Materials and equipment that contain or have come in contact with these organisms must be disinfected or sterilized by autoclaving prior to disposal, and work surfaces decontaminated. (ii) Cotton plugged pipettes may be used where the hazard is minimal, but mechanical pipetting devices are preferable and are required for more hazardous material. (iii) Pointed syringe needles should be avoided where possible. (iv) Eating, drinking, and smoking are not permitted in the work area. (v) Laboratory personnel should wash hands before and after experiments involving these materials. (vi) Laboratory doors must be closed while such experiments are in progress. (vii) Appropriate clothing such as clean laboratory coats, or similar apparel, and closed shoes should be worn when handling potentially hazardous organisms (cf.ref.12). Laboratory coats should not be worn outside the work area. (viii) Each laboratory should have an effective insect- and rodent-control program. (ix) Any research group working with agents with a known or potential biohazard should have an emergency plan, including a clean-up procedure to follow if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (x) If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. (xi) Serological monitoring, where appropriate, should be provided for.

B. Physical containment levels - A variety of combinations (levels) of special procedures, equipment, and laboratory installations that provide additional physical barriers can be formed. For example, 31 combinations are listed in "Laboratory Safety at the Center for Disease Control" (4);

four levels are associated with the "Classification of Etiologic Agents on the Basis of Hazard" (5), and with the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (3); and the National Cancer Institute uses three for research on oncogenic viruses (6). We emphasize that these are an aid to, and not a substitute for, good technique. Personnel must be competent in the effective use of all equipment needed for the required containment level as described below. We define only four levels of physical containment here, both because the accuracy with which one can presently assess the biohazards that may result from recombinant DNAs does not warrant a more detailed classification, and because additional flexibility can be obtained by combination of the physical with the biological barriers. Though different in detail, these four levels ($P1 < P2 < P3 < P4$) approximate those given for the classification of etiologic agents (i.e., classes 1 through 4; ref. 5), in the Asilomar summary statement (i.e., minimal, low, moderate, and high; ref. 3), and by the NCI (low, moderate, and high; ref. 6), as is indicated by the P-number or adjective in the following headings. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of hazardous organisms.

We anticipate, and indeed already know of, procedures (14) which enhance physical containment capability in novel ways. For example, miniaturization of screening, handling and analytical procedures provides substantial containment of a given host-vector system. Thus, such procedures should reduce the need for the standard types of physical containment.

P1 level (minimal) - Requires standard microbiological practices (see A above).

P2 level (low) - In addition to the P1 procedures, this level requires the following: (i) Access to the laboratory is controlled when handling potentially hazardous organisms requiring P2 containment, but not otherwise. During the controlled period appropriate biohazard signs should be posted at access points to the laboratory, and only authorized persons who have been advised of the potential biohazard should enter when such signs are posted. The signs may be removed upon completion of decontamination after the hazardous procedures. Experiments of less biohazard can be carried out concurrently in carefully demarcated areas of the same laboratory if necessary, given that adequate precautions are taken to avoid cross-contamination and no animals or plants are in the area.¹ (ii) Mouth pipetting is prohibited; mechanical pipetting devices are required. (iii) Specific precautions are required for those procedures that have a high potential for release of aerosols containing potentially hazardous material, e.g., centrifugation should be carried out in airtight cups or rotors (8); sonication and blending should be similarly contained or carried out in appropriate enclosed biological safety cabinets. (iv) All potentially hazardous material must be inactivated before it enters the drainage system.

P3 level (moderate) - In addition to the P2 procedures, this level requires the following: (i) Operations with potentially hazardous organisms

¹ For example, interaction between vectors containing cloned DNA fragments and permissive host strains used in the same laboratory during cell growth, storage, or disposal might lead to escape from the biological containment.

that require P3 containment should be carried out in a laboratory that is separated from areas where less than P3 containment is practiced. The ventilation system shall be balanced to provide for an inflow of supply air from the adjacent corridor into the laboratory through the access doorway. This supply air flow shall be in excess of 50 cubic feet per minute (15). The general exhaust air from these laboratories should be discharged outdoors and dispersed to the atmosphere to prevent re-entry into the building. If recirculated, the air must be decontaminated. Appropriate biohazard signs should be posted at access points to the laboratory, and only persons specifically authorized by the principal investigator should enter when these signs are posted. Normally, authorized persons should be limited to those who work in the laboratory.

(ii) Properly installed biological safety cabinets, meeting appropriate NIH performance standards (16), should be used for all transfer operations and for all procedures likely to produce aerosols. Operations that produce large amounts of aerosols, such as blending, sonication, vortex mixing, and vigorous agitation, should be avoided where possible. Where necessary, they must be carried out in enclosed systems that are not opened until adequate time has been allowed for aerosols to settle. (iii) Gloves should be worn during the handling of biohazardous materials. (iv) Vacuum lines should be protected by filters.

P3 level research can be conducted in laboratories where the room air flow and general exhaust air conditions described above cannot be achieved, provided that this work is contained in closed systems which are maintained under a negative pressure of at least 0.25 inches water gauge. Operations

including centrifugation, fractionation, sonication, etc., shall be contained in these systems. All materials, before removal from these systems, shall be sterilized or transferred to a sealed unbreakable container which is then removed from the system through a chemical decontamination tank, or after the entire system has been decontaminated.

P4 level (high) - Work areas are in a special facility of the type designed to contain highly infectious and hazardous microbiological agents (15). These areas are isolated by airlocks, a negative pressure environment, personnel clothing change and shower rooms, and treatment systems to inactivate or remove biohazardous agents contaminating exhaust air, liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the facility. The handling of the biohazardous agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters.

C. Biological containment levels - Biological barriers are specific to each host-vector system. Hence the criteria for this mechanism of containment cannot be generalized to the same extent as for physical containment. This is particularly true at the present time when our experience with existing host-vector systems and our predictive knowledge about projected systems is sparse. The classification of experiments with recombinant DNAs that is necessary for the construction of the experimental guidelines (Section III) can be accomplished with least confusion if we use the host-vector system as the primary element and the source of the inserted DNA as the secondary element in the classification. It is therefore convenient

to specify the nature of the biological containment under host-vector headings such as those given below for Escherichia coli K-12 (pp. 13-25).

D. Publication - The committee strongly recommends that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures practiced, to aid and forewarn others who might consider repeating the work.

III. Experimental Guidelines

A general rule that, though obvious, deserves statement is that the level of containment required for any experiment on DNA recombinants shall never be less than that required for the most hazardous component used to construct and clone the recombinant DNA (i.e., vector, host, and inserted DNA). In most cases the level of containment will be greater, particularly when the recombinant DNA is formed from species that ordinarily do not exchange genetic information. Handling the purified DNA will generally require less stringent precautions than will propagating the DNA. However, the DNA itself should be handled at least as carefully as one would handle the most dangerous of the DNAs used to make it.

The above rule by itself effectively precludes certain experiments--namely, those in which one of the components is in Class 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (5), as these are excluded from the United States by law and USDA administrative policy. There are additional experiments which may engender such serious biohazards that they should not be performed at this time. These are considered prior to presentation of the containment guidelines for permissible experiments.

A. Experiments that should not be performed - We recognize that it can be argued that certain of the recombinants placed in this category could be adequately contained at this time. Nonetheless, our estimates of the possible dangers that may ensue if that containment fails are of such a magnitude that we consider it the wisest policy to at least defer experiments on these recombinant DNAs until there is more information to accurately assess that danger and to allow the construction of more effective biological barriers. In this respect, these guidelines are more stringent than those initially recommended (1).

We therefore strongly advise that the following experiments not be initiated at the present time: (i) Cloning of recombinant DNAs derived from the pathogenic organisms in Classes 3, 4, and 5 of "Classification of Etiologic Agents on the Basis of Hazard" (5), regardless of the vector-host system used. (ii) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins of very high toxicity (e.g., botulinum or diphtheria toxins). (iii) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase virulence and host range. (iv) Widespread or uncontrollable release into the environment of any organism containing a recombinant DNA molecule unless a series of controlled tests leave no reasonable doubt of safety. (v) Transfer of drug resistance traits to microorganisms that are not known to acquire them naturally should be deferred if they could compromise the use of a drug to control disease agents in medicine or agriculture.

In addition, we recommend that at this time large-scale experiments (e.g., more than 10 liters of culture) with recombinant DNAs known to

make harmful products not be carried out. We differentiate between small- and large-scale experiments with such DNAs because the probability of escape from containment barriers normally increases with increasing scale. However, specific experiments in this category that are of direct societal benefit may be excepted from this rule if special biological containment precautions and equipment designed for large-scale operations are used, and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee of the NIH.

B. Containment guidelines for permissible experiments - It is anticipated that most recombinant DNA experiments initiated before these guidelines are next reviewed (i.e., within the year) will employ E. coli K-12 host-vector systems. These are also the systems for which we have the most experience and knowledge regarding the effectiveness of the containment provided by existing hosts and vectors necessary for the construction of more effective biological barriers.

For these reasons, E. coli K-12 appears to be the system of choice at this time, although we have carefully considered arguments that many of the potential dangers are compounded by using an organism as intimately connected with a man as is E. coli. Thus, while proceeding cautiously with E. coli, serious efforts should be made toward developing alternate host-vector systems; this subject is discussed in considerable detail in Appendix A.

We therefore consider DNA recombinants in E. coli K-12 before proceeding to other host-vector systems.

1. Biological containment criteria using E. coli K-12 host-vectors

EK1 host vectors - These are host-vector systems that can be estimated to already provide a moderate level of containment, and include most of the presently available systems. The host is always E. coli K-12, and the vectors include nonconjugative plasmids [e.g., pSC101, ColE1 or derivatives thereof (17-24)] and variants of bacteriophage λ (25-27).

The E. coli K-12 nonconjugative plasmid system is taken as an example to illustrate the approximate level of containment referred to here. The available data from experiments involving the feeding of bacteria to humans and calves (28-30) indicate that E. coli K-12 did not usually colonize the normal bowel, and exhibited little, if any, multiplication while passing through the alimentary tract even after feeding high doses (i.e., 10^9 to 10^{10} bacteria per human or calf). However, general extrapolation of these results may not be warranted because the implantation of bacteria into the intestinal tract depends on a number of parameters, such as the nature of the intestinal flora present in a given individual and the physiological state of the inoculum. Moreover, since viable E. coli K-12 can be found in the feces after humans are fed 10^7 bacteria in broth (28) or 3×10^4 bacteria protected by suspension in milk (29), transductional and conjugational transfer of the plasmid vectors from E. coli K-12 to resident bacteria in the fecal matter before and after excretion must also be considered.

The nonconjugative plasmid vectors cannot promote their own transfer, but require the presence of a conjugative plasmid for mobilization and transfer to other bacteria. When present in the same cell with derepressed conjugative plasmids such as F or R1d_{rd}19, the non-conjugative ColE1, ColE1-trp and pSC101 plasmids are transferred to suitable recipient strains under ideal laboratory conditions at frequencies of about $0.5 \cdot 10^{-4}$ to 10^{-5} , and 10^{-6} per donor cell, respectively. These frequencies are reduced by another factor of 10^2 to 10^4 if the conjugative plasmid employed is repressed with respect to expression of donor fertility.

The experimental transfer system which most closely resembles nonconjugative plasmid transfer in nature is a triparental mating. In such matings, the bacterial cell possessing the nonconjugative plasmid must first acquire a conjugative plasmid from another cell before it can transfer the nonconjugative plasmid to a secondary recipient. With ColE1, the frequencies of transfer are 10^{-2} and 10^{-4} to 10^{-5} when using conjugative plasmid donors possessing derepressed and repressed plasmids, respectively. Mobilization of ColE1-trp and pSC101 under similar laboratory conditions is so low as to be usually undetectable (31). Since most conjugative plasmids in nature are repressed for expression of donor fertility, the frequency at which nonconjugative plasmids are mobilized and transferred by this sequence of events in vivo

is difficult to estimate. However, in calves fed on an antibiotic-supplemented diet, it has been estimated that such triparental nonconjugative R plasmid transfer occurs at frequencies of no more than 10^{-10} to 10^{-12} per 24 hours per calf (30). In terms of considering other means for plasmid transmission in nature, it should be noted that transduction does operate in vivo for Staphylococcus aureus (32) and probably for E. coli as well. However, no data are available to indicate the frequencies of plasmid transfer in vivo by either transduction or transformation.

These observations indicate the low probabilities for possible dissemination of such plasmid vectors by accidental ingestion, which would probably involve only a few hundred or thousand bacteria provided that at least the standard practices (Section II-A above) are followed, particularly the avoidance of mouth pipetting. The possibility of colonization and hence of transfer are increased, however, if the normal flora in the bowel is disrupted by, for example, antibiotic therapy (33). For this reason, persons receiving such therapy should not work with DNA recombinants formed with any E. coli K-12 host-vector system during the therapy period and for seven days thereafter; similarly, persons who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work, as should those who require large doses of antacids.

The observations on the fate of E. coli K-12 in the human alimentary tract are also relevant to the containment of recombinant DNA formed with bacteriophage λ variants. Bacteriophage can escape from the laboratory either as mature infectious phage particles or in bacterial host cells in which the phage genome is carried as a plasmid or prophage. The fate of E. coli K-12 host cells carrying the phage genome as a plasmid or prophage is similar to that for plasmid-containing host cells as discussed above. The survival of the λ phage genome when released as infectious particles depends on their stability in nature, their infectivity and on the probability of subsequent encounters with naturally occurring λ -sensitive E. coli strains. Although the probability of survival of λ and its infection of resident intestinal E. coli in animals and humans has not been measured, it is estimated to be small given the high sensitivity of λ to the low pH of the stomach, the insusceptibility to λ infection of smooth E. coli cells (the type that normally resides in the gut), the infrequency of naturally occurring λ -sensitive E. coli (34) and the failure to detect infective λ particles in human feces after ingestion of up to 10^{11} λ particles (35). Moreover, λ particles are very sensitive to dessication.

Establishment of λ as a stable lysogen is a frequent event (10^0 to 10^{-1}) for the att⁺ int⁺ cI⁺ phage so that this mode of escape would be the preponderant laboratory hazard;

however, most EK1 λ vectors currently in use lack the att and int functions (25-27) thus reducing the probability of lysogenization to about 10^{-5} to 10^{-6} (36-38). The frequency for the conversion of λ to a plasmid state for persistence and replication is also only about 10^{-6} (39). Moreover, the routine treatment of phage lysates with chloroform (40) should eliminate all surviving bacteria including lysogens and λ plasmid carriers. Lysogenization could also occur when an infectious λ containing cloned DNA infects a λ -sensitive cell in nature, and recombines with a resident lambdoid prophage. Although λ -sensitive E. coli strains seem to be rare, a significant fraction do carry lambdoid prophages (41-42) and thus this route of escape should be considered.

While not exact, the estimates for containment afforded by using these host-vectors are at least as accurate as those for physical containment, and are sufficient to indicate that currently employed plasmid and λ vector systems provide a moderate level of biological containment. Other nonconjugative plasmids and bacteriophages that, in association with E. coli K-12 can be estimated to provide the same approximate level of moderate containment are included in the EK1 class.

EK2 host-vectors - These are host-vector systems that have been genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. The genetic modifications of the E. coli K-12 host and/or the plasmid or phage vector should not permit survival of the cloned DNA fragment in other than specially designed and carefully regulated laboratory environments at a frequency greater than 10^{-8} . This absolute measure of biological containment has been selected because it is a realistic measurable entity. Indeed, by testing the contributions of preexisting and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible to substantially indicate, if not prove, that the specially designed host-vector system can provide a margin of biological containment in excess of that required.

For EK2 plasmid vectors, no more than one in 10^8 host cells containing a chimeric plasmid should be able to perpetuate the cloned DNA fragment under nonpermissive conditions designed to represent the natural environment either by survival of the original host or as a consequence of transmission of the cloned DNA by transformation, transduction or conjugation to a host with properties common to those in the natural environment.

In terms of potential EK2 plasmid-host systems, the following types of genetic modifications should reduce survival of the cloned DNA. The examples given are for illustrative purposes and should

not be construed to encompass all possibilities. The presence of the non-conjugative plasmids ColE1-trp and pSC101 in an E. coli K-12 strain possessing a mutation eliminating host-controlled restriction and modification (hsdS) results in about 10^3 -fold reduction in mobilization to restriction-proficient recipients. The combination of the dapD8, Δ bioH-asd and Δ gal-chl^r mutations in E. coli K-12 results in no detectable survivors in feces of rats following feeding by stomach tube of 10^{10} cells in milk and similarly leads to complete lysis of culture suspended in broth medium lacking diaminopimelic acid. E. coli K-12 strains with Δ thyA and deoC(dra) mutations undergo thymineless death in growth medium lacking thymine and give a 10^5 -fold reduced survival during passage through the rat intestine compared to wild-type thy⁺ E. coli K-12. (However, the Δ thyA mutation alone or in combination with a deoB(drm) mutation only reduces in vivo survival by a factor of 10^2 .) Other host mutations, as yet untested, that might further reduce survival of the plasmid-host system or reduce plasmid transmission are: the combination polA(TS) recA(TS) Δ thyA which might interfere with ColE1 replication and lead to DNA degradation at body temperatures; Con⁻ mutations that reduce the ability of conjugative plasmids to enter the plasmid-host complex and thus should reduce mobilization of the cloned DNA to other strains; and mutations that confer resistance to known transducing phages.

Mutations can also be introduced into the plasmid to cause it to be dependent on a specific host, to make its replication thermosensitive and/or to endow it with a killer capability such that all cells (other than its host) into which it might be transferred will not survive.

In the construction of EK2 plasmid-host systems it is important to use the most stable mutations available, preferably deletions. Obviously, the presence of all mutations contributing to higher degrees of biological containment must be verified periodically by appropriate tests. In testing the level of biological containment afforded by a proposed EK2 plasmid-host system, it is important to design relevant tests to evaluate the survival of the cloned DNA under conditions that are possible in nature and that are also most advantageous for its perpetuation. For example, one might conduct a triparental mating with a primary donor possessing a derepressed F-type or I-type conjugative plasmid, the safer host with $\Delta\text{bioH-asd}$, dapD8 , $\Delta\text{gal-chl}^{\text{r}}$, ΔthyA , deoC , trp and hsdS mutations and a plasmid vector carrying an easily detectable inserted gene such as for ampicillin resistance or trp^+ , and a secondary recipient that is Su^+ hsdS trp (i.e., permissive for the recombinant plasmid). Such matings would be conducted in a medium lacking diaminopimelic acid and thymine and survival of the Ap^{r} or trp^+ marker in any of the three strains followed as a function of time. Survival of the cloned marker by transduction could also be evaluated by introducing a known generalized transducing phage

into the system. Similar experiments should also be done using a secondary recipient that is restrictive for the recombinant plasmid as well as with primary donors possessing repressed conjugative plasmids with incompatibility group properties like those commonly found in enteric microorganisms. Since a common route of escape of plasmid-host systems in the laboratory might be by accidental ingestion, it is suggested that the same types of experiments be conducted in suitable animal-model systems. In addition to these tests on survival of the cloned DNA, it would be useful to determine the survival of the host strain under nongrowth conditions such as in water and as a function of drying time after a culture has been spilled on a lab bench.

For EK2 phage vectors, no more than one in 10^8 recombinant phage particles should be able to perpetuate the cloned DNA fragment under non-permissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring the cloned DNA to a host (or its resident lambdoid prophage) with properties common to those in the natural environment.

In terms of potential EK2 λ -host systems, the following types of genetic modification should reduce survival of the cloned DNA. The examples given are for illustrative purposes

and should not be construed to encompass all possibilities.

The probability of establishing λ lysogeny in the normal laboratory host should be reduced by removal of the phage att site, the Int function, the repressor gene(s) and adding virulence-enhancing mutations. The frequency of plasmid formation, although normally already less than 10^{-6} , could be further reduced by defects in the p_R-Q region, including mutations such as vir-s, cro(TS), c17, ri^C, O(TS), P(TS), and nin. Moreover, chloroform treatment used routinely following cell lysis would reduce the number of surviving cells, including possible lysogens or plasmid carriers, by more than 10^8 . The host may also be modified by deletion of the host λ att site and inclusion of one or more of the mutations described above for plasmid-host systems to further reduce the chance of formation and survival of any lysogen or plasmid carrier cell.

The survival of escaping phage and the chance of encountering a sensitive host in nature are very low, as discussed for EK1 systems. The infectivity of the phage particles could be further reduced by introducing mutations (e.g., suppressed ambers) which would make the phage particles extremely unstable except under special laboratory conditions (e.g., high concentrations of salts or putrescine). Another means would be to make the phage itself a two-component system, by eliminating the tail genes and reproducing the

phage as heads packed with DNA; when necessary and under specially controlled conditions, these heads could be made infective by adding tail preparations. An additional safety factor in this regimen is the extreme instability of the heads, unless they are stored in 10M putrescine, a condition easy to obtain in the laboratory but not in nature. The propagation of the escaping phage in nature could further be blocked by adding various conditional mutations which would permit growth only under special laboratory conditions or in a special permissive laboratory host with suppressor or *gro*-type (*mop*, *anaB*, *rpoB*) mutations. An additional safety feature would be the use of an $r^- m^-$ (*hsdS*) laboratory host, which produces phage with unmodified DNA which should be restricted in $r^+ m^+$ bacteria that are probably prevalent in nature. The likelihood of recombination between the λ vector and lambdoid prophages which are present in some *E. coli* strains might be reduced by elimination of the Red function and the presence of the recombination-reducing Gam function together with mutations contributing to the high lethality of the λ phage. However, these second-order precautions might not be relevant if the stability and infectivity of the escaping λ particles are reduced by special mutations or by propagating the highly unstable heads.

Despite multiple mutations in the phage vectors and laboratory hosts, the yield of phage particles under suitable laboratory conditions should be high (10^{10} - 10^{11} particles/ml).

This permits phage propagation in relatively small volumes and constitutes an additional safety feature.

The phenotypes and genetic stabilities of the mutations and chromosome alterations included in these λ -host systems indicate that containment well in excess of the required 10^{-8} or lower survival frequency for the cloned DNA fragment should be attained. Obviously the presence of all mutations contributing to this high degree of biological containment must be verified periodically by appropriate tests. Laboratory tests should be performed with the bacterial host to measure all possible routes of escape of cloned DNA such as the frequency of lysogen formation, the frequency of plasmid formation and the survival of the lysogen or carrier bacterium. Similarly, the potential for perpetuation of the cloned DNA fragment carried by infectious phage particles could be tested by challenging typical wild-type *E. coli* strains or a λ -sensitive nonpermissive laboratory K-12 strain, especially one lysogenic for a lambdoid phage.

In view of the fact that accurate assessment of the probabilities for escape of infectious λ grown on $r^- m^- Su^+$ hosts is dependent upon the frequencies of r^- , Su^+ , and λ -sensitive strains in nature, investigators are encouraged to screen *E. coli* strains for these properties. These data will also be useful in predicting frequencies of successful escape of plasmid cloning vectors harbored in $r^- m^- Su^+$ strains.

When any investigator has obtained data on the level of containment provided by a proposed EK2 system, these should be reported as rapidly as possible to permit general awareness and evaluation of the safety features of the new system. Investigators are also encouraged to make such new safer cloning systems generally available to other scientists. NIH should take appropriate steps to aid in the distribution of these safer vectors and hosts.

EK3 host-vectors - These are EK2 systems for which the specified containment shown by laboratory tests has been independently confirmed by appropriate tests in animals, including humans or primates, and in other relevant environments in order to provide additional data to validate the levels of containment afforded by the EK2 vectors and hosts. Evaluation of the effects of individual or combinations of mutations contributing to the biological containment should be performed as a means to confirm the degree of safety provided and to further advance the technology of developing even safer vectors and hosts.

2. Classification of experiments using the E. coli K-12 containment systems

In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimums. It is recommended that higher levels of biological containment (EK3 > EK2 > EK1) be used if they are available and are equally appropriate for the purposes of the experiment.

<a> Shotgun Experiments

These experiments involve the production of recombinant DNAs between the vector and the total DNA or (preferably) any partially purified fraction thereof from the specified cellular source.

(i) Eukaryotic DNA recombinants

Primates - P3 physical containment + an EK3 host-vector, or P4 physical containment + an EK2 host-vector, except for DNA from embryonic tissue or primary tissue cultures therefrom, and germ-line cells for which P3 physical containment + an EK2 host-vector can be used. The basis for the lower estimated hazard in the case of DNA from the latter tissues is their relative freedom from horizontally acquired adventitious viruses.

Other mammals - P3 physical containment + an EK2 host-vector.

Birds - P3 physical containment + an EK2 host-vector.

Cold-blooded vertebrates - P2 physical containment + an EK2 host-vector except for embryonic or germ-line DNA which require P2 physical containment + an EK1 host-vector.

Other cold-blooded animals and lower eukaryotes - P2 physical containment + an EK1 host-vector. If the eukaryote in this class is a known pathogen (i.e., an agent listed in Class 2 of ref. 5 or a plant pathogen) or carries such an agent, the containment should be increased to P3 + EK2.

Higher plants - P2 physical containment + an EK2 host-vector. If the plant carries a known pathogenic agent or makes a product known to be dangerous to any species, the containment should be raised to P3 physical containment + an EK2 host-vector.

(ii) Prokaryotic DNA recombinantsProkaryotes that exchange genetic information with *E. coli*² -

The level of physical containment is directly determined by the rule of the most dangerous component (see introduction to Section III). Thus P1 conditions can be used for DNAs from those bacteria in Class 1 of ref. 5 ("Agents of no or minimal hazard....") which naturally exchange genes with *E. coli*; and P2 conditions should be used for such bacteria if they fall in Class 2 of ref. 5 ("Agents of ordinary potential hazard...."), or are plant pathogens. EK1 host-vectors can be used for all experiments requiring only P1 physical containment; in fact, experiments in this category can be performed with *E. coli* K-12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. Experiments with DNA from species requiring P2 physical containment which are of low pathogenicity (for example, enteropathogenic *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*) can use EK1 host-vectors, but those of moderate pathogenicity (for example, *Salmonella typhi*, *Shigella dysenteriae* type I, and *Vibrio cholerae*) should use EK2 host-vectors.³

² Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection and/or conjugation with transfer of phage, plasmid and/or chromosomal genetic information.

³ The bacteria which constitute Class 2 of ref. 5 ("Agents of ordinary potential hazard....") represent a broad spectrum of etiologic agents which possess different levels of virulence and degrees of communicability. We think it appropriate for our specific purpose to further subdivide the agents of Class 2 into those which we believe to be of relatively low pathogenicity and those which are moderately pathogenic. The several specific examples given may suffice to illustrate the principle. The Committee has asked the Center for Disease Control to help prepare a complete list of Class 2 agents subdivided into low and moderate pathogenicity that could be distributed to interested parties.

Prokaryotes that do not exchange genetic information with

E. coli - The minimum containment conditions for this class consist of P2 physical containment + an EK1 host-vector, and apply when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal. Experiments with DNAs from pathogenic species (Class 2 ref. 5 plus plant pathogens) should use P3 + EK2.

Experiments extending the range of resistance to therapeutically useful drugs and disinfectants should use P2 + EK2 containment or higher depending on the virulence of the donor.

(iii) Characterized clones of DNA recombinants derived from
shotgun experiments

When a cloned DNA recombinant has been rigorously characterized⁴ and there is sufficient evidence that it is free of harmful genes,⁴ then experiments involving this recombinant DNA can be carried out under P1 + EK1 conditions if the inserted DNA is from a species that exchanges genes with E. coli, and under P2 + EK1 conditions if not.

⁴The terms "characterized" and "free of harmful genes" are unavoidably vague. But, in this instance, before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the granting agency. Such approval would be contingent upon data concerning: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector.

 Purified cellular DNAs other than plasmids, bacteriophages,
and other viruses

The formation of DNA recombinants from cellular DNAs that have been enriched⁵ by physical and chemical techniques (i.e., not by cloning) and which are free of harmful genes can be carried out under lower containment conditions than used for the corresponding shotgun experiment. In general, the containment can be decreased one step in physical containment (P4 → P3 → P2 → P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (EK3 → EK2 → EK1) while maintaining the specified physical containment--provided that the new condition is not less than that specified above for characterized clones from shotgun experiments (Section <a>--iii).

<c> Plasmids, bacteriophages, and other viruses

Recombinants formed between EK-type vectors and other plasmid or virus DNAs have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombinants

⁵ A DNA preparation is defined as enriched if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation. The reason for lowering the containment level when this degree of enrichment has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10⁵-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

in E. coli K-12 hosts. They do not apply to other hosts where they may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

(i) Animal viruses

P4 + EK2 or P3 + EK3 should be used to isolate DNA recombinants that include all or part of the genome of an animal virus. This recommendation applies not only to experiments of the "shotgun" type but also to those involving partially characterized subgenomic segments of viral DNAs (for example, the genome of defective viruses, DNA fragments isolated after treatment of viral genomes with restriction enzymes, etc.). When cloned recombinants have been shown by suitable biochemical and biological tests to be free of harmful regions, they can be handled in P3 + EK2 conditions. In the case of DNA viruses, harmless regions include the late region of the genome; in the case of DNA copies of RNA viruses, they might include the genes coding for capsid proteins or envelope proteins.

(ii) Plant viruses

P3 + EK1 or P2 + EK2 conditions should be used to form DNA recombinants that include all or part of the genome of a plant virus.

(iii) Eukaryotic organelle DNAs

The containment conditions given below apply only when the organelle DNA has been purified.⁶ Mitochondrial DNA from primates:

⁶ The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

P3 + EK1 or P2 + EK2. Mitochondrial or chloroplast DNA from other eukaryotes: P2 + EK1. Otherwise, the conditions given under shotgun experiments apply.

(iv) Prokaryotic plasmid and phage DNAs

Plasmids and phage from hosts that exchange genetic

information with E. coli - Experiments with DNA recombinants

formed from plasmids or phage genomes that have not been characterized with regard to presence of harmful genes or are known to contribute significantly to the pathogenicity of their normal hosts should use the containment conditions specified for shotgun experiments with DNAs from the respective host. If the DNA recombinants are formed from plasmids or phage that are known not to contain harmful genes, or from purified⁷ and characterized plasmid or phage DNA segments known not to contain harmful genes, the experiments can be performed with P1 physical containment + an EK1 host-vector.

Plasmids and phage from hosts that do not exchange genetic

information with E. coli - The rules for shotgun experi-

ments with DNA from the host apply to their plasmids or phages, with the following qualifications. Experiments with DNA recombinants formed with foreign plasmids or phage containing genes which extend the range of resistance of the recipient to therapeutically useful drugs should use P3 physical containment + an EK2 host-vector. The minimum containment

⁷ The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was achieved or verified by more than one procedure.

conditions for this category (P2 + EK1) can be used for plasmid and phage, or for purified⁸ and characterized segments of plasmid and phage DNAs, when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal.

NOTE: Where applicable, cDNAs (i.e., complementary DNAs) synthesized in vitro from cellular or viral RNAs are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under <a>, shotgun experiments; cDNAs formed from purified and characterized RNAs are included under ; cDNAs formed from viral RNAs are included under <c>; etc.

3. Experiments with other prokaryotic host-vectors

Other prokaryotic host-vector systems are at the speculative, planning, or developmental stage, and consequently do not warrant detailed treatment here at this time. However, the containment criteria for different types of DNA recombinants formed with E. coli K-12 host-vectors can, with the aid of some general principles given here, serve as a guide for containment conditions with other host-vectors when appropriate adjustment is made for their different habitats and characteristics.

⁸

The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was achieved or verified by more than one procedure.

In general, the strain of any prokaryotic species used as the host should conform to the definition of Class 1 etiologic agents given in ref. 5 (i.e., "Agents of no or minimal hazard...."), and the plasmid or phage vector should not make the host more hazardous. In addition, it is recommended that the newly developed host-vector systems offer some distinct advantage over the E. coli K-12 host-vectors--for instance, thermophilic organisms or other host-vectors whose major habitats do not include humans and/or economically important animals and plants. Appendix A gives a detailed discussion of the B. subtilis system, the most promising alternative to date.

At the initial stage, the host-vector should exhibit at least a moderate level of biological containment comparable to EK1 systems, and be capable of modification to obtain high levels of containment comparable to EK2 and EK3. The type of confirmation test(s) required to move a host-vector from an EK2-type classification to an EK3-type will clearly depend upon the preponderant habitat of the host-vector. For example, if the unmodified host-vector propagates mostly in, on, or around higher plants, but not appreciably in warm-blooded animals, modification should be designed to reduce the probability that the host-vector can escape to and propagate in, on, or around such plants, or transmit recombinant DNA to other bacterial hosts that are able to occupy these ecological niches, and it is these lower probabilities which should be confirmed. The following principles should be followed in using the containment criteria given for experiments with E. coli K-12 host-vectors as a guide for other prokaryotic systems. Experiments with DNA from prokaryotes (and their plasmids or viruses) should be classified according to whether the prokaryote in question

exchanges genetic information with the host-vector or not, and the containment conditions given for these two classes with E. coli K-12 host-vectors applied. Transfer of recombinant DNA to plant pathogens can be made safer by using nonreverting, doubly auxotrophic, non-pathogenic variants. Experiments using a plant pathogen that affects elements of a local flora will require more stringent containment than if carried out in areas where they are not common.

Experiments with DNAs from eukaryotes (ant their plasmids or viruses) can also follow the criteria for the corresponding experiments with E. coli K-12 vectors if the major habitats of the given host-vector overlap those of E. coli. If the host-vector has a major habitat that does not overlap those of E. coli (e.g., root nodules in plants), then the containment conditions for some eukaryotic recombinant DNAs should be increased (for instance, higher plants and their viruses in the preceding example), while others may be reduced.

4. Experiments with eukaryotic host-vectors

<a> Animal host-vector systems - Because host cell lines generally have little if any capacity for propagation outside the laboratory, the primary focus for containment is the vector, although cells should also be derived from cultures expected to be of minimal hazard. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by humans; thus vectors

should be chosen that have little or no ability to replicate in human cells. To be used as a vector in a eukaryotic host, a DNA molecule should display all of the following properties:

(1) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture.

(2) Its functional anatomy should be known--that is, there should be a clear idea of the location within the molecule of:

- a) the sites at which DNA synthesis originates and terminates,
- b) the sites that are cleaved by restriction endonucleases,
- c) the template regions for the major gene products.

(3) It should be well studied genetically. It is desirable that mutants be available in adequate number and variety, and that quantitative studies of recombination have been performed.

(4) The recombinant should be defective, that is, its propagation as a virus is dependent upon the presence of a complementing helper genome. This helper should either (a) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector to that particular cell line) or (b) consist of a defective genome or an appropriate conditional lethal mutant virus (in which case the experiments would be done under non-permissive conditions), making vector and helper dependent upon each other for propagation. However, if

none of these is available, the use of a non-defective genome as helper would be acceptable.

Currently only two viral DNAs can be considered as meeting these requirements: these are the genomes of polyoma virus and SV40.

Of these, polyoma virus is highly to be preferred. SV40 is known to propagate in human cells, both in vivo and in vitro, and to infect laboratory personnel, as evidenced by the frequency of their conversion to producing SV40 antibodies. Also, SV40 and related viruses have been found in association with certain human neurological and malignant diseases. SV40 shares many properties, and gives complementation, with the common human papova viruses. By contrast, there is no evidence that polyoma infects humans, nor does it replicate to any significant extent in human cells in vitro. However, this system still needs to be studied more extensively. Appendix B gives further details and documentation.

Taking account of all these factors, it is proposed that:

1. Polyoma Virus

- a Recombinant DNA molecules consisting of defective polyoma virus genomes plus DNA sequences of any non-pathogenic organism, including Class 1 viruses (5), can be propagated in or used to transform cultured cells in P3 conditions; appropriate helper virus can be used if needed. Whenever there is a choice, it is urged

that mouse cells, derived preferably from embryos, be used as the source of eukaryotic DNA. Polyoma virus is a mouse virus and recombinant DNA molecules containing both viral and cellular sequences are already known to be present in virus stocks grown at a high multiplicity. Thus, recombinants formed in vitro between polyoma virus DNA and mouse DNA are presumably not novel from an evolutionary point of view.

- b. Such experiments can be done, under P4 conditions, if the recombinant DNA contains segments of the genomes of Class 2 animal viruses (5). Once it has been shown by suitable biochemical and biological tests that the cloned recombinant contains only harmless regions of the viral genome (see Section IIIB-2-c-i) and that the host range of the polyoma virus vector has not been altered, experiments can be continued under P3 conditions.

2. SV40 Virus

- a. Defective SV40 genomes, with appropriate helper, can be used in P4 conditions as a vector for recombinant DNA molecules containing sequences of any non-pathogenic organism or Class I virus (5), (i.e., a shotgun type experiment); established lines of cultured cells should be used.
- b. Such experiments can be carried out in P3 conditions if the non-SV40 DNA segment is (a) a purified⁹ segment of prokaryotic DNA lacking toxigenic genes, or (b) a segment

⁹The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was achieved or verified by more than one procedure.

of eukaryotic DNA whose function has been established, which does not code for a toxic product, and which has been previously cloned in a prokaryotic host-vector system.

- c. A recombinant DNA molecule consisting of defective SV40 DNA lacking substantial segments of the late region, plus DNA from non-pathogenic organisms or Class I viruses (5), can be propagated as an autonomous cellular element in established lines of cells under P3 conditions provided that there is no exogenous or endogenous helper, and that it is demonstrated that no infectious virus particles are being produced. Until this has been demonstrated, the appropriate containment conditions specified in 2. a. and 2. b. shall be used.
- d. Recombinant DNA molecules consisting of defective SV40 DNA and sequences from non-pathogenic prokaryotic or eukaryotic organisms or Class I viruses (5) can be used to transform established lines of non-permissive cells under P3 conditions. It must be demonstrated that no infectious virus particles are being produced; rescue of SV40 from such transformed cells by co-cultivation or transfection techniques must be carried out in P4 conditions.

- 3. Efforts should be made to ensure that all cell lines are free of virus particles and mycoplasma.

Since SV40 and polyoma are limited in their scope to act as vectors, chiefly because the amount of foreign DNA

that the normal virions can carry probably cannot exceed 2×10^6 daltons, we urge that consideration be given to the development of systems in which recombinants can be cloned and propagated purely in the form of DNA, rather than in the coats of infectious agents. Plasmid forms of viral genomes or organelle DNA should be explored as possible cloning vehicles in eukaryotic cells.

** Plant host-vector systems**

Cells in tissue cultures, seedlings, or plant parts (e.g., tubers, stems, fruits, and detached leaves) or whole mature plants of small species (e.g., Arabidopsis) can be handled under the P1-P4 containment conditions that we have specified previously. However, work in most plants poses additional problems. P2 physical containment conditions can be provided by: (i) the best insect-proof greenhouses, (ii) appropriate disinfection of contaminated plants, pots, soil, and runoff water, and (iii) adoption of the other standard practices for microbiological work. P3 physical containment can be sufficiently approximated by confining the operations with whole plants to growth chambers like those used for work with radioactive isotopes, provided that (i) such chambers are modified to produce a negative pressure environment with the exhaust air appropriately filtered, and (ii) that other operations with infectious materials are carried out under the specified P3 conditions. The P2 and P3 conditions specified earlier are therefore extended to include these cases for work on higher plants.

The host cells for experiments on recombinant DNAs may be cells in culture, in seedling or plant parts, or in whole plants. Cells in whole plants that cannot be adequately contained should not be used as hosts for shotgun experiments at this time, and attempts to infect whole plants with DNA recombinants cloned elsewhere should not be initiated until their effects on host cells in culture, seedlings, or plant parts have been studied.

Organelle or plasmid DNAs or DNAs of viruses of low pathogenicity to plants may be used as vectors. In general, the same preference criteria for selecting host-vectors given in the preceding section on animal systems apply to plant systems, where organelle and plasmid DNAs can be grouped together as offering the potential of highly contained vectors that should be investigated.

Experiments on recombinant DNAs formed between the initial moderately contained vectors and DNA from cells of species in which the vector DNA can replicate, either autonomously or as an integrated segment of the cell's genome, should use P2 physical containment--provided that the source of the DNA is itself not pathogenic or known to carry pathogenic agents, or to produce products dangerous to plants. In the latter cases, of if the vector is an unmodified virus of low pathogenicity, the experiments should be carried out under P3 conditions.

Experiments on recombinant DNAs formed between the above vectors and DNAs from other species can also be carried out under P2 if that DNA has been purified¹⁰ and determined not to contain harmful genes. Other-

¹⁰The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was achieved or verified by more than one procedure.

wise, the experiments should be carried out under P3 conditions if the source of the inserted DNA is not itself a pathogen, or known to carry such pathogenic agents, or to produce harmful products--and under P4 conditions if these conditions are not met.

The development and use of host-vector systems that exhibit a high level of biological containment permit a decrease of one step in the physical containment specified above (P4 → P3 → P2 → P1).

<c> Fungal or similar lower eukaryotic host-vector systems

The containment criteria for experiments on recombinant DNAs using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, in that the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. We therefore consider that the containment guidelines given for experiments with E. coli K-12 and other prokaryotic host-vectors (Sections IIIB-1 and -2, respectively) provide adequate direction for experiments with these lower eukaryotic host-vectors. This is particularly true at this time since the development of these host-vectors is presently in the speculative stage.

IV. Implementation

A. Role of the principal investigator - The principal investigator has the responsibility for estimating the potential biohazards associated with the experiments on recombinant DNAs performed in the laboratories under her or his direction, for instituting the appropriate safeguards within these laboratories, for developing procedures for minimizing the effects of accidents, for training and ensuring the proficiency of relevant personnel

in the application of these safeguards and procedures, for informing personnel of both the potential hazards and the basis on which these hazards have been estimated, and for maintaining these practices on a continuing basis.

Appropriate monitoring of personnel (including, where appropriate, serological monitoring and checking of intestinal flora) should be conducted frequently. Evidence presented to this committee has suggested that E. coli chromosomal markers such as nalidixic acid resistance (nal^r) and the inability to ferment arabinose (ara⁻) or mannitol (mtl⁻) are useful for monitoring the presence of a host organism in the laboratory environment and in laboratory personnel. In addition, we recommend that NIH designate a data collection group to which principal investigators must report any laboratory incident involving a failure of P3 or P4 containment or any serious or extended illness of a worker.

The experimental guidelines given here are to help the principal investigator determine the nature of the safeguards that should be implemented for experiments with different types of recombinant DNAs. Because the complexity of types is great, these guidelines are bound to be incomplete in some regards. Hence they are not meant to substitute for the investigator's own evaluation of the containment conditions required for each experiment. Whenever this evaluation calls for an increase in containment over that indicated in the guidelines, the investigator has the responsibility for instituting such an increase. By contrast, the containment conditions should not be decreased over

those called for in the guidelines without peer review (see Section IV.B). In the event of a question as to what containment conditions should apply, the Recombinant DNA Molecule Program Advisory Committee may be petitioned for a reevaluation of the level of containment required.

The institution of appropriate safeguards includes a continuing control on containment procedures, on the effective operation of the physical containment facilities, and on those aspects of the genotype of the host-vectors that are relevant to the biological containment they provide. Although the data on the phenotypic characteristics of a given host-vector that determine its level of biological containment usually will have been obtained in other laboratories, the principal investigator has the responsibility for being able to justify the overall containment determination on the basis of such data. That is, it is not sufficient that he or she simply be assured by some other person of the determination of the containment level; rather investigators should be or become sufficiently knowledgeable to make their own determination. In addition, investigators have the responsibility of ascertaining that the hosts and/or vectors exhibit the required genotype prior to their use in experiments with DNA recombinants in their laboratories. Such ascertainment generally involves a simple phenotypic test for each relevant mutation.

B. Institutional role - Implementation starts with the principal investigator's evaluation of the potential biohazards associated with a given project and of the appropriate safeguards to be applied. To help in this

evaluation and application, each institution or group of institutions where research on DNA recombinants takes place should form a biohazard committee that would have the following two functions. The first and most useful function would be to serve as a source of advice and references regarding: (i) the availability and quality of the safety equipment and laboratory installation modes required for P3 and P4 physical containment, (ii) the availability and level of biological containment of different kinds of host-vector systems, (iii) suitable training of personnel, and (iv) more general data on the potential biohazards associated with different types of recombinant DNAs. To this end, each local biohazard committee should create a central reference file and library of catalogues, books, articles, newsletters, and other communications relevant to the above subjects.

The second, more formal function of this committee would be to examine the equipment and installations in laboratories requiring P3 or P4 physical containment, and, if they meet the requirements for such containment, to so certify. It is not the responsibility of the local committee to determine either the scientific quality or the containment conditions required for a given project.

When investigators apply to an agency for funds to support research projects involving recombinant DNAs, or whenever they decide to initiate or significantly change research on recombinant DNAs under existing grants, they should provide the agency with the above certification for the facility or plans for the facility (if the research requires P3 or P4 physical containment) along with a statement containing their

evaluation of the potential biohazards and the containment conditions they will use for the proposed projects.

The peer group reviewing the scientific merit of a research proposal for the agency will then make an independent evaluation of the potential biohazards and determine whether the proposed containment conditions provide the appropriate safeguards, using the guidelines given here as their basic reference. If the review group concludes that the safeguards are appropriate, the grant would be processed on the basis of scientific merit in the usual fashion.

If there is some question concerning the adequacy of the containment capability that cannot be resolved at this level, then the matter may be referred to an appropriate committee.

In those cases where the investigator wishes to initiate or significantly change research on recombinant DNAs under existing grants, the agency can, without peer review, make the decision to endorse this initiation or change. However, if there is some question regarding the adequacy of the containment capability that cannot be resolved at this level, the matter should be referred to the initial review group.

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Statement on the use of *Bacillus subtilis* in recombinant molecule technology

Unquestionably, *Escherichia coli* is the most well characterized unicellular organism. Years of basic research have enabled investigators to develop a well characterized genetic map, to obtain detailed knowledge of virulent and temperate bacteriophages, and to explore the physiology, genetics, and regulation of plasmids. More recently, the development of DNA-mediated transformation has permitted exogenous fragments or molecules of DNA to be incorporated into the genome or to reside as self-replicating units. The discovery of transformation of *Bacillus subtilis* by Spizizen (1) stimulated the development of an alternative model system. The purpose of this report is to summarize the current status of this genetic system and to describe the actual and potential vectors and vehicles available for recombinant molecule technology.

A. Current knowledge of the chromosomal architecture and mechanisms of genetic exchange in *B. subtilis*

Two mechanisms of genetic exchange have been utilized to establish the linkage map of *B. subtilis*, DNA-mediated transformation (capable of transferring approximately 1% of the genome) and transduction with bacteriophage PBS1 (capable of transferring 5-8% of the chromosome). Recent detailed genetic studies with PBS1 by Lepesant-Kejzlorová et al. (2) have resulted in the development of a circular genetic map for this organism. The current edition of the map (3) contains 196 loci. Biophysical analyses

have established that the chromosome is circular (4) and replicates bidirectionally (5).

Transformation with purified fragments of DNA is a highly efficient process in B. subtilis with frequencies of 1 to 4% usually attained for any auxotrophic or antibiotic resistance markers. Frequencies of approximately 10% transformation can be achieved with DNA prepared from gently lysed L-forms or protoplasts (6). These large fragments of DNA are readily incorporated by the recipient cell. Generalized transduction occurs with bacteriophages SP10 (7), PBS1 (8), and SPP1 (9), while a low frequency of specialized transduction has been reported with bacteriophage ϕ 105 (10).

Although transformation is most efficient in homologous crosses (B. subtilis into B. subtilis), it has also been possible to exchange DNA among closely related species (11). The most extensively studied members of the B. subtilis genospecies include B. licheniformis, B. pumilus, B. amyloliquefaciens, and B. globigii (refer to reference 12 for a review and references 13-15 for examples of this heterologous exchange). This exchange occurs even though there is a surprisingly wide discrepancy between DNA - DNA hybridization among these organisms (16). Even though the frequency of transformation is low in the heterologous cross [e.g., B. amyloliquefaciens (donor)/B. subtilis (recipient)], the newly acquired DNA from B. amyloliquefaciens in the B. subtilis background can be readily transferred at high efficiencies to other recipient strains of B. subtilis (14). Therefore, the extremely high frequency of transformation permits the recognition and selection of rare events.

B. Current and potential vectors for recombinant molecule experiments

Lovett and coworkers have recently described cryptic plasmids in B. pumilus (17) and B. subtilis (18). Of these organisms, B. subtilis ATCC 7003 appears to be the most useful since it carries one to two copies of a plasmid with a molecular weight of 46×10^6 . This strain is also closely related to B. subtilis 168. Another strain of B. subtilis (ATCC 15841) contains 16 copies of a plasmid with a molecular weight of 4.6×10^6 . Currently it is not known whether genetic markers can be readily introduced into these plasmids. To date it has not been possible to readily stabilize plasmids derived from B. pumilus in B. subtilis even with heavy selective pressure (P. Lovett, personal communication).

Two temperate bacteriophages are under development as vectors in B. subtilis, $\phi 3T$ and SP02. Lysogeny of thymine auxotrophs (strains carrying thyA thyB) by bacteriophage $\phi 3T$ results in "conversion" to a Thy^+ phenotype. The attachment site for this bacteriophage and the bacteriophage gene for thymidylate synthetase (thyP) map between the bacterial thyA and thyB loci in the terminal region of the chromosome of B. subtilis (19). The viral genome is readily cleaved by the site-specific endonuclease, Bam I (20), to produce 5 fragments (one of which carries the thyP gene). The thyP carrying gene can be integrated into the bacterial genome in the absence of the intact viral genome. Because deletions are available that include the thyP region, it is theoretically possible to introduce thyP at many sites on the chromosome. The thyP gene can be readily purified

for insertion into plasmids or utilized as a scaffold to integrate other heterologous DNA into the chromosome of B. subtilis. Alternatively, it is possible to purify fragments of the chromosome by gel electrophoresis (21, 22), for insertion into bacteriophage ϕ 3T or SP02. At present, unfortunately, only the former carries a selective marker, i.e., the gene for thymidylate synthetase, thyP.

C. Development of vehicles

B. subtilis is a Gram-positive sporulating rod that usually inhabits soil. Although it can exist on cutaneous surfaces of man (23) and experimental animals, it rarely produces disease. To develop a suitable vehicle it is imperative to have a host that is asporogenic. The most appropriate deletion mutation is deletion 29 (cit D). In addition to a deficiency in sporulation this mutant rapidly lyses when it has reached the end of its growth cycle. Presumably this is due to the failure to inactivate one of the autolytic enzymes (24). Through the introduction of a D-alanine requirement (34 ug/ml) it is possible to block transport of compounds that are transported by active transport (25,26). The further introduction of thymine auxotrophy (defects in the thyA thyB loci) will enable the strain to survive only with a plasmid vector carrying the purified thyP gene from bacteriophage ϕ 3T or a defective bacteriophage ϕ 3T carrying the thyP gene but attached to the chromosome at an alternative site (due to the presence of deletion 29 in the host). We have recently isolated temperature-sensitive thyP mutants. If we can isolate a temperature-dependent lysogen that will grow only at 48°C it should be possible to make an unusual vehicle.

D. Site-specific endonucleases

Recently two restriction modification systems have been observed between B. subtilis 168 and other bacilli. Trautner et al. have isolated an effective system that inhibits infection of the R strain of B. subtilis by bacteriophage SPPI propagated on B. subtilis 168 (27). The site-specific nuclease recognizes the sequence GGCC. Young, Radnay, and Wilson CCGG observed a restriction modification between B. amyloliquefaciens and B. subtilis 168 (28). The endonuclease from B. amyloliquefaciens (20) recognizes the sequence GGATCC (29). More recently, two additional enzymes have CCTAGG been isolated from B. globigii (30). The recognition sequence is not known.

E. Advantages and liabilities of the B. subtilis system

a. Advantages

1. B. subtilis is nonpathogenic. Asporogenic deletion mutants are available to preclude the problem of persistence through sporulation.
2. The circular chromosomal map is well defined. At least 196 loci have been positioned.
3. The organism is commercially important in the fermentation industry.
4. Large numbers of organisms can be disposed of readily with minimal environmental impact.
5. Unlike E. coli, it lacks endotoxin in the cell wall. Therefore the cells can be used as a single cell protein source.
6. The frequency of transformation is very high, facilitating the detection of rare events.

7. A unique bacteriophage, $\phi 3T$, exists that carries a gene that can be readily purified for "scaffolding" experiments.

b. Disadvantages

1. The knowledge of genetics and physiology of plasmids and viruses is primitive compared with E. coli.

2. High-frequency, specialized transduction is not available as a means of gene enrichment.

Based on its promise, it seems appropriate, and not chauvinistic, to urge development of this system.

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Polyoma and SV40 Virus

Polyoma virus is a virus of mice, and infection of wild mouse populations is a common event, for the virus has often been isolated from a high proportion of healthy adult animals, both wild and laboratory bred, of many colonies (Gross, L., Proc. Soc. Exp. Biol. 88, 362-368, 1955; Rowe, W. P., Bact. Rev. 25, 18-31, 1961). As far as is known the virus almost never causes a disease in these animals. However, when large quantities of the virus are inoculated into newborn or suckling mice or hamsters, a variety of solid tumors is induced (Gross, L., Oncogenic Viruses, Second Edition, Pergamon Press, NY).

Polyoma virus grows lytically in mouse cells in tissue culture. Thus mouse cells probably in culture are transformed only by virus particles that contain certain kinds of defective genomes. Cells of other rodent species, however, can be transformed by polyoma virus particles that contain complete genomes (Folk, W., J. Virol., 11, 424-431, 1973). The virus does not replicate to a significant extent in human cells in tissue culture (Eddy, B.E., Virol. Monogr., 7, 1-114, 1969; Pollack, R. E., Salas, J., Wang, R., Kusano, T., and Green, H., J. Cell Physiol. 77, 117-120, 1971). The resistance of the cells seems to be a consequence of the failure of the virus to absorb or uncoat. However even when naked viral DNA is introduced into the cells only an abortive cycle of replication ensues; early viral proteins are made, there is induction of cellular DNA synthesis, but no expression of late viral proteins is detectable (Gruen, R., Grassmann, M. and Grassmann, A., Virology, 58, 290-293, 1974).

There is no evidence that polyoma virus can infect humans (Hartley, J., Huebner, R., Parker, J. and Rowe, W. P., unpublished data). Thus no antibodies to the virus have been detected in people living in buildings that are infested with virus-infected mice, nor in laboratory workers who have been exposed to the virus for a number of years.

At most, a small segment of polyoma virus DNA shows weak homology with a portion of the late region of SV40 DNA (Ferguson, J. and Davis, R. W., J. Mol. Biol., 94, 135-150, 1975). However, there appears to be no genetic interaction between the two viruses and there is no immunological cross-reaction between the gene products of the two viruses.

SV40 causes persistent but apparently harmless infections of the kidneys of virtually all adult rhesus monkeys (Hsiung, G. D., Bact. Revs. 32, 185-205, 1968), it causes tumors when injected into newborn hamsters (Girardi, A. J., Sweet, B. H., Slotnick, V. B. and Hillemann, M. R., Proc. Soc. Exp. Biol. Med., 105, 420-427, 1964) and transforms cells of several mammalian species (including human). SV40 is able to infect humans since antibodies to the virus are found in a small proportion of the human population (Shah, K. V., Goverdhan, M. K. and Ozer, H. L., Am. J. Epid. 93, 291-298, 1970) and serum conversions have been noted in many laboratory personnel who have been exposed to the virus (Horvath, L. B., Acta Microbiol. Acta Sci. Hung. 12, 201-206, 1965).

Isolations of SV40 have been reported from humans, twice from patients suffering from the rare demyelinating disease, progressive multifocal leukoencephalopathy (Weiner, L., Herndon, R., Narayan, O., Johnson, R. T., Shah, K., Rubinstein, L. G., Preziosi, T. J. and Conley, F. K., New England

J. Med. 286, 385-390, 1972) and apparently from a tumor of a person with metastatic melanoma (Soriano, F., Shelburne, C. E. and Gokcen, M., Nature, 249, 421-424, 1974). In other studies a non-structural antigen characteristic of papovaviruses, T antigen, has been detected in the nuclei of cells cultured from 2 meningiomas, while another SV40-specific antigen, U antigen, has been found in the cells of a third tumor of the same type (Weiss, A. F., Portman, R., Fisher, H., Simon, J. and Zang, K. D., Proc. Nat. Acad. Sci. USA 72, 609-613, 1975). Furthermore new papovaviruses have been isolated from the brains of patients with PML (JC virus - Padgett, B. L., Walker, D. L., zuRhein, G. M., Eckroade, R. I. and Dessel, B. H., Lancet 1, 1257-1260, 1971), from the urine of a patient carrying a renal allograft (BK virus - Gardner, S. D., Field, A. M., Coleman, D. V. and Hulme, B. Lancet 1, 1253-1257, 1971) and from a reticulum cell sarcoma and the urine of patients with the sex-linked recessive disorder, Wiskott-Aldrich syndrome (Takemoto, K. K., Rabson, A. S., Mullarkey, M. F., Blaese, R. M. Garon, C. F. and Nelson, D. J., Nat. Cancer Inst., 53, 1205-1207, 1974). All of these viruses which are distributed widely throughout human populations share antigenic and biological properties with SV 40; the virus particles are identical in size and architecture (Madeley, C. R., In Virus Morphology, Churchill-Livingstone, London, 134-135, 1972); the non-structural intracellular T antigen, which appears to be coded by the A gene of SV40 cross reacts extensively with antigens found in cells infected or transformed by BK or JC viruses; both JC and BK viruses induce tumors in newborn hamsters (Walter, D. L., Padgett, B. L., zuRhein, B. M., Albert, A. E. and Marsh, R. F., Science 181, 674-676,

1973; Shah, K. V., Daniel, R. W. and Strandberg, J., J. Nat. Cancer Inst. 54, 945-950, 1975); BK virus causes transformation of hamster cells in culture (Major, E.D., and DiMayorca, G., Proc. Nat. Acad. Sci. US, 70, 3210-3212, 1973; Portolani, M., Barbanti, A., Brodano, G. and LaPlaca, M.J., Virology 15, 420-422, 1975) and is able to complement the growth of certain temperature-sensitive mutants of SV40 (Masion, B. H. and Takemoto, K. K., submitted for publication).

Further Work

At present, a potential eukaryotic vector of choice is polyoma virus. And while available information indicates that it fulfills all the necessary criteria, we recommend that the following subjects be further investigated:

1. The molecular mechanism of resistance of human cells to the virus.
2. The extent of homology between polyoma virus DNA and the DNAs of human papovaviruses.
3. The ability of human papovaviruses to complement defective polyoma virus genomes.

Summary of the Workshop on the DESIGN AND TESTING OF
SAFER PROKARYOTIC VEHICLES AND BACTERIAL HOSTS FOR
RESEARCH ON RECOMBINANT DNA MOLECULES

Torrey Pines Inn, La Jolla, California

The development of techniques for the cloning of DNA from both prokaryotic and eukaryotic organisms in bacteria has had great impact on research in biology and medicine and promises extraordinary social benefits. The biohazards involved in the use of this technology in many instances is very difficult to assess. For this reason codes of practice are being formulated in the United States and other countries for the conduct of those experiments that present a potential biohazard. One of the requirements for conducting certain cloning experiments is the use of safer vector (bacteriophage or plasmid) -host systems, i.e., vector-bacterium systems that have restricted capacity to survive outside of controlled conditions in the laboratory. Approximately sixty scientists from the United States and several foreign countries participated in a workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules at La Jolla, California, on 1 to 3 December, 1975. The workshop was sponsored by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. The purposes of the meeting were the exchange of recent data on the development of safer prokaryotic host-vector systems, devising methods of testing the level of containment provided by these systems and exploring the various directions that future research should take in the construction of safer bacterial systems for the cloning of foreign DNA.

The first session of the workshop, chaired by W. Szybalski (University of Wisconsin), was devoted to bacteriophage vectors. Szybalski outlined the main safety features of the two-component, phage-bacterial system, in which the host bacteria offer the safety feature of not carrying the cloned DNA, and the phage vectors cannot be propagated in the absence of an appropriate host. There are two primary escape routes for the clones of foreign DNA carried by the phage vector: (1) establishment of a stable prophage or plasmid in the laboratory host used for phage propagation, and subsequent escape of this self replicating lysogen or carrier system, and (2) escape of the phage vector which carries the cloned DNA and its subsequent productive encounter with a suitable host in the natural environment. The general concensus was that to ensure safety, both routes should be blocked by appropriate genetic modifications. For phage λ , route (1) can be blocked by phage mutations that interfere with lysogenization (att^- , int^- , cI^- , $cIII^-$, vir^-) and plasmid formation (N^+ , $ninR$, vS , ri^c , $cl7$, Ots , $crots$), and by mutations on the *Escherichia coli* host that affect these processes ($attB^-$, $dnaA$ ts) and host survival. Route (2), [which is of low probability since λ phages do not survive well in natural environments (no λ CI phage was recovered after ingestion of 10^8 - 10^{11} particles), are killed by desiccation, and have a low chance to encounter a naturally sensitive host] can be blocked further by the following phage modifications: (a) mutations which result in extreme instability of the infectious phage particles under all conditions other than those specially designed for phage propagation in the laboratory (e.g., high concentrations of putrescine or some other compound), or (b) employing phage vectors in which the tail genes are deleted and which permit propagation of only the DNA-packed heads; only under laboratory conditions could such heads be made transiently infectious by rejoining them with separately prepared tails. The high instability of the phage would minimize the possibility of

transfer of the cloned genes into receptive bacteria found in nature. Moreover, the propagation of the phage can be blocked by many conditional mutations, which would be designed to block any secondary route of escape, mainly depending on transfer of the cloned DNA into another phage or bacterial host. It was recommended further that the vector be designed in such a manner as to permit easy insertion and monitoring of the foreign DNA and rapid assay of the safety features and give a high yield of cloned DNA (not less than 10^{11} molecules per ml). There also was general agreement that host-phage systems other than *E. coli* should be considered, especially those restricted to very rare and unusual environments. Also, plasmids derived from phage vectors and which give very high DNA yields while exhibiting safety features, e.g., λ dvcrts, should be considered as vehicles for cloned DNA.

Szybalski and S. Brenner (Cambridge University) stressed that research on recombinant DNA molecules may lend itself to very simple and inexpensive mechanical containment, e.g., a small sealed glove box, since all the vectors that carry such recombinant molecules possibly can be both created and destroyed in such a box, while development of special methods might permit study of many properties of the recombinant DNA, without ever removing it from the box.

These safety features were reflected in the subsequent presentations. F. Blattner and W. Williams (University of Wisconsin) described four specially constructed λ - ϕ 80 phages which incorporate many of these safety features, and which they named Charon phages, for the mythical boatman of the river Styx. Some of these highly contained phages give yields of over 10^{11} particles/ml. R. Davis, J. Cameron and K. Struhl (Stanford University) found that λ phages that carry foreign DNA never grow as well as the parental vector, which would select against their survival in the nature. They also reported that some eukaryotic genes could be expressed in *E. coli*, partially compensating for deficiencies in the histidine pathway or in *polA* or *lig* functions. These investigators surveyed over 1000 strains of *E. coli* isolated in the natural environment and did not find a single strain that could support propagation of the λ vir vector.

V. Bode (Kansas State University) discussed the possibility of growing tail-free λ heads. Such heads, which are packed with DNA, are very fragile, unless stored in 0.01 M putrescine buffer. Head yields close to 10^{11} /ml could easily be attained and, when required, heads could be quantitatively rejoined with separately supplied tails under special laboratory conditions. W. Arber, D. Scandella and J. Elliott (University of Basel) described bacterial host mutants that permit efficient infection only by phages with a full complement of DNA. This permits selecting for vectors that carry long fragments of foreign DNA.

K. Matsubara, T. Mukai and Y. Takagi (University of Osaka and Kyushu University), and G. Hobom and P. Philippson (University of Freiburg and Stanford University) described various defective λ plasmids (λ dv) that could be used as efficient vectors. Matsubara has shown that temperature-sensitive *cro* mutations permit obtaining between 1000 and 3000 cloned molecules per cell and at the same time result in killing of the carrier cells at body temperature. The mutations *Ots* and *Pts* were also evaluated as safety features. Philippson described many new λ dv plasmids created by cutting λ DNA with *HindIII* and *BamI* restriction endonucleases followed by ligation. The final talk by F. Young, G. Wilson and M. Williams (University of Rochester) summarized the progress on the development of safer *Bacillus subtilis* host mutants and phages, especially ϕ 3, as vectors. New restriction nucleases, Bgl-1 and Bgl-2, were also described.

The morning session on bacteriophage vectors was followed by a session on plasmid vectors that was chaired by D. Helinski (University of California, San Diego). Helinski presented the following properties as highly desirable characteristics of a safer plasmid vehicle: (a) non-conjugative; (b) non-mobilizable or poorly mobilizable by a conjugative plasmid; (c) possesses little or no extraneous genetic information; (d) poorly recombines or does not recombine with the chromosome of the host cell; (e) provides no selective advantage to the host cell or the selective property is conditional; and (f) possesses mutations that restrict its maintenance to a specific host, prevent replication at mammalian body temperature and/or provide the plasmid with the capability of killing any cell to which it might be transmitted other than the host cell. V. Hershfield (University of California, San Diego) described the properties of a variety of derivatives of the ColE1 plasmid and the broad-host range, P-type plasmid, RK2. One of the ColE1 derivatives, ColE1-*trp*, constructed in collaboration with C. Yanofsky and N. Franklin (Stanford University) provides the means to use the tryptophan genes of *E. coli* as a selective marker in transformation with recombinant DNA in situations where it is desirable to avoid antibiotic resistance genes. In addition, Hershfield described collaborative work with H. Boyer that resulted in the development of a mini-ColE1 plasmid and derivatives of this plasmid (mini-ColE1-*kan* and mini-ColE1-*trp*) as cloning vehicles. Finally, she described the temperature-sensitivity properties of *trp* and *kan* derivatives of a temperature-sensitive replication mutant of ColE1 isolated by J. Collins (Molecular Biology Institute, Stockholm) and hybrid ColE1 plasmids carrying the *EcoRI* generated Cts fragment of bacteriophage λ -trp61.

J. Carbon (University of California, Santa Barbara) described a replica plating method that greatly facilitates the detection of *E. coli* clones bearing ColE1 plasmids. The procedure, which utilizes the F₁ plasmid to promote the transfer of a hybrid ColE1 plasmid to a suitable auxotrophic recipient, was successful in identifying clones bearing hybrid plasmids carrying a number of different regions of the *E. coli* chromosome. The contributions of A. J. Clark and collaborators (University of California, Berkeley) were relevant to the problem of the mobilization and subsequent transfer of non-conjugative plasmids carrying foreign DNA of a potentially hazardous nature. Clark described the variations in transmission frequencies between the non-conjugative plasmids pSC101, pML31, pSC138 and a number of pSC101 hybrids containing various *EcoRI* fragments of F when the conjugal transfer of these plasmids was promoted by several different conjugative plasmids.

I. C. Gunsalus and collaborators (University of Illinois) and A. Chakrabarty (General Electric Research and Development Center) described the properties of a variety of plasmids isolated from *Pseudomonas putida*. These contributions were followed by a discussion on the merits of developing plasmid-host systems involving *Pseudomonas* strains that naturally exhibit unusual growth requirements. Similar studies with plasmids isolated from *Bacillus megaterium* by B. Carlton (University of Georgia) from *B. subtilis* by P. Lovett (University of Maryland) and other naturally occurring *Bacillus* species by W. Goebel and K. Bernhard (Microbiology Institute, Wurzburg) were discussed and their further development as plasmid-host cloning systems was explored. It was clear from these presentations that considerable progress has been made recently in the identification and characterization of a variety of plasmid elements that occur naturally in *Pseudomonas* and *Bacillus* species. Several of the plasmids described show considerable promise as plasmid cloning systems involving a host other than *E. coli*.

A third session on the ecology and epidemiology of vector-host systems was chaired by S. Falkow (University of Washington). This workshop emerged, in part, from expressed fears that microorganisms containing cloned fragments of foreign DNA may potentially pose a threat to health or disrupt the normal ecological chain in some manner. Consequently, this session was devoted to a review of currently available information on the ecology and epidemiology of *E. coli* and related bacterial species since it was recognized that *E. coli* K-12 would be the prokaryotic host most commonly employed in the cloning of DNA molecules in the immediate future.

F. Ørskov (Escherichia Reference Center, Copenhagen) reviewed the state of *E. coli* serotyping and what has been learned about the distribution of *E. coli* types in health and disease. Only certain *E. coli* types are generally recognized as good colonizers of the human gut and such strains come from a handful of the 160 well defined O (lipopolysaccharide) antigen types and invariably possess K (acidic polysaccharide capsule) antigens. Some serotypes apparently have become disseminated worldwide and possibly represent the proliferation of a bacterial clone because of, as yet unknown, selective pressures. In contrast, *E. coli* K-12 has no detectable O or K antigens and is considered to be rough. This may account, at least in part, for its demonstrable poor ability to colonize the human or animal gut. However, R. Freter (University of Michigan) pointed out that we still remain largely ignorant of the factors which control intestinal *E. coli* populations. Freter also noted that while adherence to the mucosal surface of the small intestine is important in the pathogenesis of *E. coli* diarrheal disease, the 'normal' long-lasting symbiotic relationship between a mammalian host and bacterium is established in the cecum and colon. It is in these locations that factors come into play to determine whether an *E. coli* strain passing through the intestine will become successfully implanted or whether it will be quickly eliminated in the feces. The factors controlling implantation include competition for substrates, inhibitors and the physiological state of the organism when it reaches the large bowel. For example, ingested *E. coli* previously grown under usual laboratory conditions fare poorly while cells of the same strain 'pre-adapted' in Eh, pH, etc., often colonize well. Freter has developed a continuous flow culture model which may be useful in studying the mechanisms of implantation. Falkow reviewed the pathogenicity of *E. coli*. *E. coli* causes diarrheal disease either by direct invasion of the bowel epithelium or by elaboration of enterotoxin(s). While invasive *E. coli* appear to owe their pathogenicity to a constellation of at least five unlinked chromosomal gene clusters, toxigenic *E. coli* species generally owe their pathogenicity to the possession of two species, Ent and K. The introduction of Ent and K plasmids may be sufficient to convert a normal wild-type *E. coli* into a strain now capable of causing overt clinical disease. However, the introduction of these plasmids into *E. coli* K-12 sublines had no discernable effect on their ability to cause disease, although the K-12 strains could now better colonize calves. Despite the observation that *E. coli* K-12 did not appear to offer a significant hazard as a potential enteric pathogen even when it possessed well-defined determinants of pathogenicity it was emphasized by Ørskov, Freter and Falkow that *E. coli* K-12 strains carrying recombinant DNA molecules could still act as effective genetic donors *in vivo* and still posed a significant problem requiring control. E. Geldreich (U.S. Environmental Protection Agency, Cincinnati, Ohio) discussed the possible outcomes of the release of *E. coli* containing recombinant DNA molecules into the aquatic environment and concluded that total reliance cannot be placed on sewage treatment and the natural self-purification capacity of receiving waters to limit potential hazards. While these are realistic barriers to the dissemination of *E. coli* and associated fecal organisms via the water route, they are not infallible because of technological limitations, improper operational practices and system overloading. Finally, M. Starr (University

of California, Davis) described the numerous genera of gram-negative bacteria found naturally occurring in the soil and on plants. He stated that most of these organisms do not appear to be a reasonable alternative to *E. coli* K-12 as a host for recombinant DNA molecules. Indeed, Starr pointed out that since such genera as *Erwinia*, *Rhizobium* and *Agrobacterium* are known to conjugate with *E. coli*, the potential dissemination of recombinant DNA molecule includes a greater spectrum of microorganisms than just enteric species.

The fourth session of the workshop, chaired by R. Curtiss III (University of Alabama), was concerned with the construction of safer bacterial hosts for DNA cloning. The goals in constructing safer host strains enumerated at the beginning of the session included introduction of mutations that would: (a) preclude colonization in normal ecological niches; (b) preclude cell wall biosynthesis except in specially defined media; (c) cause degradation of genetic information in normal ecological niches; (d) cause vectors to be host-dependent; (e) minimize transmission of recombinant DNA to other strains in normal ecological niches; (f) increase usefulness for recombinant DNA molecule research; and (g) permit monitoring.

Most of the progress in developing safer hosts has been achieved with *E. coli* K-12, although F. Young described a *B. subtilis* strain with a deletion for sporulation genes which readily undergoes autolysis. The strain also has defects in genes for purine and TTP biosynthesis and a mutation conferring a D-alanine requirement can be introduced to cause cell wall biosynthesis to be defective. This strain may be defective in transformation, however, and therefore might be useful only with a phage vector which has yet to be developed and/or discovered.

A. I. Bukhari (Cold Spring Harbor Laboratory) described the use of the *dapD8* mutation in *E. coli* K-12 to block cell wall biosynthesis and another non-reverting mutation which causes sensitivity to bile salts and detergents. The *dapD8* allele is the most stable *dap* point mutation known, although it does revert at frequencies of 10^{-8} to 10^{-9} . The mutation conferring bile salts sensitivity was obtained after Mu-1 infection of an Hfr strain and, although exhibiting the theoretically useful properties of ease of DNA isolation and inability to survive in the intestinal tract, might be due to Mu insertion which would compromise its use for safe strain construction.

Curtiss reported on the work performed by him and his coworkers in constructing and testing numerous strains with different mutations. Survival of strains *in vivo* was tested by feeding rats 10^{10} cells in milk by stomach tube. Δpur mutations did not reduce strain titers in feces whereas $\Delta thyA$; $\Delta thyA\ drm$; and $\Delta thyA\ dra$ mutations gave 10^2 -fold, 10^2 -fold and 10^5 -fold reductions, respectively, in strain titers in feces. Strains with $\Delta thyA$ mutations also exhibited thymineless death in *in vitro* tests. Since strains with the *dapD8* allele can revert to Dap^+ , strains were constructed with both the *dapD8* and $\Delta bioH\ asd$ mutations. These strains have not been observed to revert to Dap^+ but can survive passage through the rat intestine and in growth media lacking diaminopimelic acid but containing NaCl and 0.5% usable carbon sources. This survival was due to the production of the mucopolysaccharide, colanic acid, which permits many of the cells to grow and survive as spheroplasts. A $\Delta gal\ chl^r$ mutation (also deletes *latt*, *bio* and *uvrB* genes) was introduced which blocks colanic acid biosynthesis and leads to no detectable survivors in media lacking diaminopimelic acid or following passage through the rat intestine. The *dapD8* $\Delta bioH\ asd$ $\Delta gal\ chl^r$ strains are more readily lysed, transform at higher frequencies

and are conjugation-defective in matings with donors possessing conjugative plasmids in the P, W and O incompatibility groups but Con^+ as recipients for F, I and T group plasmids when compared to the $\text{dap}^+ \text{gal}^+$ parent strain. Strains with *endA* mutations were also observed to exhibit increased transformation frequencies. Attempts to introduce temperature-sensitive *polA* alleles into strains to block replication of ColE1 cloning vectors at elevated temperatures and to cause DNA degradation at elevated temperatures in the presence of *recA* and ΔthyA alleles have so far been unsuccessful since the NTG-induced *polA* alleles often do not have the same properties in the constructed strains as in the strains in which the allele was originally induced. Many mutations causing a Con^- phenotype have been investigated, but many of these revert and/or do not exhibit a Con^- phenotype in matings with donors possessing conjugative plasmids of the incompatibility groups commonly found in enteric microorganisms. Some Con^- mutants exhibit increased sensitivity to bile salts; thus, the mutant described by Bukhari may also exhibit a Con^- phenotype. All of the strains constructed by the Curtiss group are SuII^+ and most have mutations abolishing restriction alone or both restriction and modification. Thus, sufficient information is now known to construct a usable safer *E. coli* K-12 host. Curtiss and collaborators are now introducing the ΔthyA and *dna* mutations into their *dapD8 \Delta\text{bioH-} \Delta\text{asd} \Delta\text{gal-} \text{chl}^r \text{-} \text{uvrB} \text{ hsr nal}^r (for ease in monitoring) $\text{Su}^+ \lambda^r \phi 80^r$ strain to accomplish this objective.*

The final session involved a general discussion of some of the major points raised previously in the workshop. There was general agreement at this session that both plasmid-host and phage-host systems have been developed that should meet the criteria of an EK2 system specified by the National Institutes of Health guidelines for research on recombinant DNA molecules. Additional testing is required to confirm the EK2 properties of these available systems, but it is anticipated that these vector-host systems will meet these tests.

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PROCEEDINGS OF A CONFERENCE ON
NIH GUIDELINES FOR RESEARCH ON
RECOMBINANT DNA MOLECULES

Public Hearings Held at a Meeting of the
Advisory Committee to the Director, NIH

February 9-10, 1976

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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P R O C E E D I N G S

The meeting was convened pursuant to notice at 9:00 a.m., Dr. Donald S. Fredrickson, Director, NIH, presiding.

DR. FREDRICKSON: Good morning. I am very glad to welcome all of you here today to provide us with your perspectives on a research activity of the National Institutes of Health that has very broad public policy implications.

This is a special meeting of the Advisory Committee to the Director of the National Institutes of Health. At the time this meeting was organized, that committee had but five members. Therefore, it was our pleasure to invite a number of former committee members, and other scientific and public representatives to participate in this special session.

There is available on the table here a number of materials which include several things that I would like to call to your attention. One is a statement from the Boston Group; several statements concerning the guidelines which all of you may wish to and should take advantage of the opportunity to see. Also there is a list of those who are members of the committee for the purposes of this meeting today.

Since many of you do not have that, I think I might take this moment to simply identify the members who are sitting at the table here today. I really prefer that the chairman try to do that rather than ask each individual to do it, at least as a test to see whether the chairman knows who is here.

(Laughter.)

At my immediate left is Dr. Lamont-Havers, who is the Deputy Director of the NIH.

Next to him is Dr. Joseph Perpich, who is the Associate Director Designate for the Office of Program Planning and Evaluation at NIH.

Skipping down to Dr. Robert Sinsheimer, who is Chairman of the Division of Biology at Cal Tech, and a molecular biologist.

Next to him is Dr. Marjorie Shaw, who is from Houston, and Director of an Institute of Medical Genetics. She is both a geneticist and a lawyer.

Skipping a chair, over to Dr. Charles Sprague, who is a cardiologist and a member of the Director's Advisory Committee. He is President of the Health Science Campus at the University of Texas at Dallas.

Dr. Marian Koshland, also a former member of this committee, is a biochemist in the department at Berkeley.

Dr. Milton Zaitlin is in the Department of Plant Pathology at Cornell University in Ithaca, a plant virologist.

Next to him is Dr. Daniel Callahan, who is Director of the Institute for Society, Ethics and Life Sciences at Hastings-on-Hudson.

Next to him is Dr. James Kelly, who is Executive Vice Chancellor at the State University of New York, and former Comptroller of the Department of HEW.

Dr. Joseph Melnick is a virologist from Baylor University in Houston, and he is also a member of the Biomedical Technology Panel of the Office of Technology Assessment.

Dr. LeRoy Walters is the Director of the Center for Bioethics at the Kennedy Institute here in Georgetown.

Directly opposite me is Dr. Robert Petersdorf, who is Chairman of the Department of Medicine, and a specialist in infectious diseases from the University of Washington in Seattle.

On his left is Mr. Peter Hutt who, for at least 5 years, was General Counsel of the Food and Drug Administration. He has just recently retired to private practice, but he is a figure who has had a tremendous amount to do with regulation in areas such as we are discussing today.

Beyond the vacant chair from Mr. Hutt is Ms. Margo Haygood, who is a student of the history of science from Harvard.

Dr. Joseph Dodds is a surgeon from Chattanooga, Tennessee, Director of the Campbell Medical Center there, and also a member of the Director's Advisory Committee, as is Dr. Petersdorf. I forgot to mention that.

Mr. Alan Ladwig is the President of the Forum for the Advancement of Students of Science and Technology here in Washington.

Next to him is Mrs. Esther Peterson, who is President of the National Consumers League, and who has served several Presidents of the United States--President Kennedy, I think, in the Department of Labor, and President Johnson as Consumer Advisor.

Professor Walter Rosenblith is Provost of Massachusetts Institute of Technology, and as an engineer and a life scientist he bridges the gaps that are represented by the problem we are talking about today.

Dr. Roy Hudson is the President of the Hampton Institute in Hampton, Virginia. He is a neuropharmacologist, by persuasion.

Next to him is Dr. Leon Jacobs, who is Associate Director for Collaborative Research at NIH.

On my right is Dr. Stetten, Deputy Director for Science at NIH, and the Chairman of the NIH Recombinant DNA Molecule Program Advisory Committee, which is the reason that we are all here today.

Good morning, Judge Bazelon. Judge David Bazelon, who is Chief Judge of the United States Court of Appeals for the District of Columbia Circuit, and Dr. Philip Handler, who is President of the National Academy of Sciences and still a Professor of Biochemistry. Good morning, gentlemen.

I think we are in full complement, and therefore ready to go.

Now, the purpose of this meeting is to seek your advice on proposed guidelines setting conditions for the conduct of certain experiments with recombinant DNA molecules. This involves the introduction of foreign genes into bacteria. We know that the promise of this research is held to be extraordinary, not only in terms of possibly opening up better knowledge of the expression of genes in cells, but also possibly moving on to the production of human products that are useful for the treatment of certain diseases, and also there is held forth a great promise of possible changes in agricultural technology.

There is also a potential risk, that microorganisms with transplanted genes might escape from the laboratory and infect human beings or animals, and alter the environment, and be dangerous and difficult to control.

Recombinant DNA research brings to the fore problems of public scrutiny of the process and the progress of basic science. That is why we are here today. These experiments are extremely technical, and they are complex, and as I am sure you members of the committee are aware from reading the materials that were sent to you, this is a rapidly moving field, and on the very leading edge of biological science.

Molecular biologists in this research area have trouble enough keeping abreast of the newest developments, and it is no surprise that scientists in other fields and the general public have difficulty in understanding the advances in recombinant research.

Yet, it is quite vital that there be public awareness and understanding of this kind of experimentation. Its theoretical promises and perils have already been much dealt with in the public press, but there is need for further informed discussion of the practical aspects of this activity.

I think it is important for you to recall that it was the scientists engaged in recombinant DNA research, including some who are here with us today--Dr. Paul Berg, Maxine Singer, and David Hogness and others--who were involved in the call for a self-imposed moratorium to assess the potential hazards and to devise appropriate guidelines.

Through their efforts, the NIH and the NSF supported the conference which was sponsored by the National Academy of Sciences and held at Asilomar in February of 1975. Their action also led the NIH to establish an Advisory Committee to develop guidelines for recombinant research that is funded by the NIH, and to devise programs for assessing and controlling hazards in such research. This NIH committee has proposed the guidelines that we will be considering at this meeting.

There is a precedent within the NIH for developing guidelines when research activity may place populations at risk. In clinical research NIH guidelines have required informed consent of human subjects, and an assessment through mechanisms such as peer review and institutional review boards of the risks and benefits of proposed research. Ethical, legal, and social values can thus be taken into account in fashioning the criteria and the standards for clinical research.

Now, the proposed guidelines on the DNA recombinant research emulate the present NIH guidelines governing clinical research, in that they represent an effort to balance scientific responsibility to the public and scientific freedom to pursue new knowledge.

The public responsibility weighs especially heavy in this genetic research area. The scientific community must have the confidence of the public, that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society.

There is a key element in achieving and maintaining this public trust, and that is that the scientific community insure an openness and candor in its proceedings. Today's meeting and the Asilomar meeting reflect an intent of science to be an open community in considering the conduct of DNA experiments.

How to insure public and scientific participation in developing national science policy is a very difficult problem, but it is not an insoluble one. Some attempts have been made in the legislature. Some others have suggested that a science court might be a possible mechanism for airing issues such as the one before us today. Its features would include

some advocacy of different opinions, some opportunity to cross-examine witnesses, and finally an opinion based on a hearing record with a statement of facts and reasoning in support of that opinion. This committee meeting is an alternative method to provide an airing of the issues, yet it incorporates some of those features that may assist us to reach a wise and proper decision.

Now, your responses to the guidelines will assist in the task of defining scientific and public interests in the research involving recombinant DNA molecules. The original agenda that you received, you will note, has been modified to allow more time for committee members to respond to or address questions to invited speakers as well as the public witnesses.

Today Dr. Berg will begin with a discussion of developments leading to the call for a moratorium of certain recombinant DNA research. Dr. Stetten will briefly review the work of the respective NIH committee, and Dr. Singer will describe the proposed NIH guidelines. Drs. Hogness and Curtiss, who are members of the NIH committee, will describe areas where there was some disagreement in drafting the guidelines. And when they have completed their brief reports, we will entertain questions that the committee members may have for the speakers.

This afternoon there will be a film describing maximum containment facilities--actually the facilities here on the Bethesda campus. This brings to our attention the very important environmental impact concerns in recombinant research; and following a description of a recombinant DNA institutional review committee, we will open the discussion to the public as well as the committee.

Questions will be entertained first from committee members and then from the public. There will then be an opportunity for statements from public witnesses who have filed their statements with us in advance. Each witness will be allowed 10 minutes. The committee members will have an opportunity to question each of the witnesses as time allows.

Tomorrow Dr. Stetten will present a summary of our discussions, and then we will welcome response from the committee and public witnesses.

In the last hour tomorrow, we will hear additional comments from you committee members on the proposed guidelines.

Now, in the next day and a half I am especially interested that you as the members of this committee provide us with a sense of your comprehension of the nature of the experiments and the risk-benefit assessment. Further, and perhaps more importantly, your response to the proposed guidelines and procedural mechanisms for monitoring this research activity will be especially helpful.

Judge Bazelon's presence here today reminds me of a quotation from his friend Felix Frankfurter, who wrote that, "The history of liberty has largely been the history of the observance of procedural safeguards." Procedural safeguards with a full exploration of relevant facts and possible alternatives must be the hallmark of the scientific process if we are to retain the trust and the wholehearted support of society. This is the challenge before us today.

If there be no questions from the committee, I propose that we take up that challenge forthwith.

Proceeding, then, to the agenda, we are going to begin with Dr. Paul Berg, Professor of Biochemistry at Stanford University Medical School, and already alluded to as one of the prime movers at the beginning of the moratorium which exists today, and about which we are discussing guidelines.

Dr. Berg, it is a pleasure to have you with us, and the forum is yours.

DR. BERG: I deeply appreciate, Dr. Fredrickson, your invitation to participate in these hearings. It is a long road to come to this room.

My purpose this morning is to try to review some of the scientific aspects of the new methodology referred to as recombinant DNA experiments, and also to try to summarize several of the potential benefits, both at the fundamental, theoretical level, as well as the practical level, and try to indicate at least some of the more obvious risks that have struck many of us who have been involved in this effort.

Then, I would like to recount a little bit about the history of how we came to the Asilomar conference, and then another colleague, Dr. Singer, will review the work that has come about since then.

Let me begin by pointing out that the past 25 years have witnessed a virtual revolution in our understanding of the structure and the workings of the genetic machinery of living cells. Although the theoretical implications of this understanding were apparent to biologists and chemists from the very beginning, the possible practical benefits of this knowledge to medicine, agriculture and industry have become clear only recently.

Now, one of the benefits that captured the imaginations of scientists and laymen alike was the notion of "genetic engineering." By that I mean the directed modification or even construction of new kinds of genetic constitutions for animals, plants and, eventually, of man. But partly because of the exaggerated and often misleading claims of the popular press, and of scientists and laymen alike, the words "genetic engineering" evoke concern as well as excitement. Because of that concern, society has expected that there would be a reckoning of the consequences before anybody would proceed with this line of experimentation.

The developments in molecular genetics during the past 10 years, but particularly in the last 3 or 4, have brought us nearer to that day of reckoning. For now, and in the next few years, we are going to be doing things that were thought to be completely improbable just a few years ago. Genes from virtually any living organism can be put into another, completely unrelated organism. For example, the genes from one species of bacteria can be transferred to a completely unrelated species of bacteria. Even more remarkable, the genes from a toad (Xenopus laevis) or a fruit fly (Drosophila melanogaster) have been introduced into the bacterium Escherichia coli, which I will refer to as E. coli.

Now, because the recipient bacterium continues to multiply, it propagates these newly acquired genes as part of its own chromosomal complement. I believe it is very likely that any kind of gene from any kind of organism--elephant, man, or whatever--could be introduced into E. coli and eventually into other organisms.

This ability to join together genetic material from any two sources and to propagate these hybrid elements in bacterial and animal cells has, I believe, produced a qualitative change in the field of genetics. I don't believe it can be argued that this is another, perhaps easier way to do what we have been doing for a long time. For now, for the first time, there is available a method which allows us to cross very large evolutionary boundaries, and to move genes between organisms that have never before had genetic contact. Consequently, we are placed in an area of biology with many unknowns, and it is our ignorance of this unknown that compels us, I believe, to pause, reflect, and to assess the implications of this line of research.

Before going on to examine the implications, I want to review briefly for you what this newly developed technique is all about. How are genetic elements from diverse organisms fused together into one chromosome and then introduced into cells where they can be propagated indefinitely? I don't propose to give you a short course in molecular biology, but let me at least summarize a few brief facts.

About 25 to 30 years ago it was established that genes are made of DNA, therefore genetic information is synonymous with chemical structure. In 1953 the DNA structure was solved, and it was clear that genes are nothing more than segments of the DNA molecule--that is, segments arranged linearly along the backbone of the double helix formed by two strands of DNA.

Normally these arrangements are conserved from one generation to the next. Occasionally DNA segments exchange between two different chromosomes, during sexual modes of reproduction. But generally this is limited to organisms of the same species.

By present techniques it is possible to cut up the DNA from different organisms in a way that allows us to reassemble new combinations of genes--that is, to construct hybrid or recombinant DNA molecules in the test tube.

The first attempts to construct hybrid DNA molecules in vitro were carried out at Stanford. David Jackson and Robert Symons in my lab, and Peter Lobban in A. Dale Kaiser's research group carried out the first such attempts. Our objective was to try to introduce new genes into animal cells in order to learn something about how gene expression is regulated in such higher organisms. To do this we explored the use of a virus, the SV40 virus, as a vector, or carrier, of the new genes to be introduced into these animal cells.

We chose SV40 because it was known that this tumor virus's DNA can be integrated into the chromosomal DNA of the recipient cells. That is, it becomes a permanent part of the genetic complement of the cells it infects.

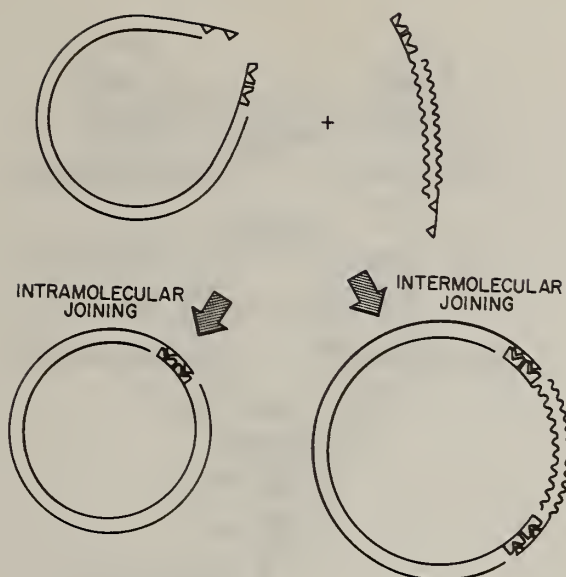
We had available to us a preparation of DNA containing the genes which code for the utilization of a sugar, galactose, derived from bacteria, E. coli. We also had large quantities of SV40 virus DNA. What we needed was a method for joining these two genetic elements together, but that had never really been done before. The principle, however, was already known to molecular biologists. It involves what has come to be known as cohesive or sticky ends.

May I have the first slide, please?

This is my simple diagram. If we look at DNA molecules, just this diagram of a double-stranded structure, I have tried to indicate these cohesive or sticky ends by little triangular lock-and-key-like structures--single-strand extensions at the ends of DNA molecules, with the chemical elements associated with them.

It was known already that two molecules which had ends that could interact with each other--that is, form complementary base pairs--could in fact be joined to produce long chains by virtue of this interaction at their cohesive or sticky ends. If a molecule, in fact, is sticky at either end, at both ends, and these are complementary to each other, these molecules can be circularized and held together in this form by the interactions of their ends.

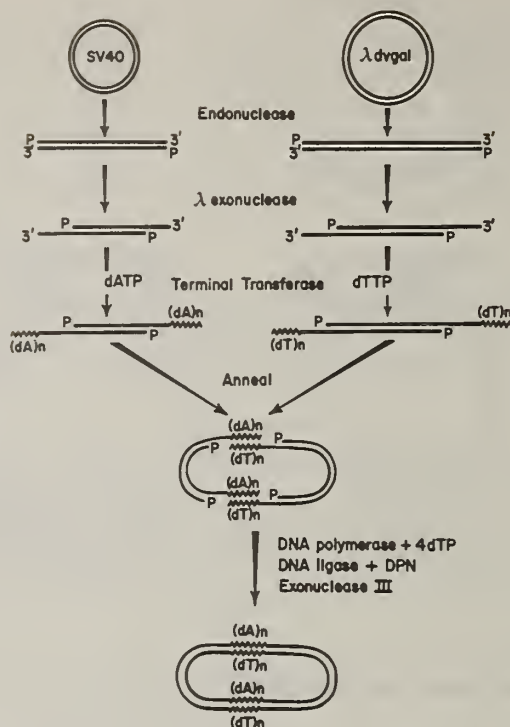
We were confronted with a somewhat different problem, because the DNA molecules we wanted to fuse together did not have naturally occurring cohesive ends. Consequently, we had to devise a procedure for generating artificial cohesive tails onto the ends of any DNA molecule.

Slide 1

May I have the next slide (2)?

I don't want to go into the technical details of the procedure; I would just like to outline it for those of you who would like to follow it. One can take two DNA molecules, cut them in such a way as to convert them into linear structures, and then by the use of appropriate enzymes modify the ends in such a way that it is possible to fuse short tails of polynucleotide sequences so that the tails of one of the linear molecules are capable of interacting with the tails of another molecule: in this particular case, tails of polydeoxy A and these molecules with polydeoxy T.

Since these ends are, in fact, cohesive with respect to each other, it is just possible to mix these two DNA molecules together and allow them to join to form this circular structure held together by these cohesive ends. Again, with the appropriate use of enzymes, which are available to us in highly purified form, it is possible to chemically join the ends of these structures, producing a chemically sealed structure that now contains the DNA elements provided by one donor and the DNA elements provided by a second donor.

Slide 2

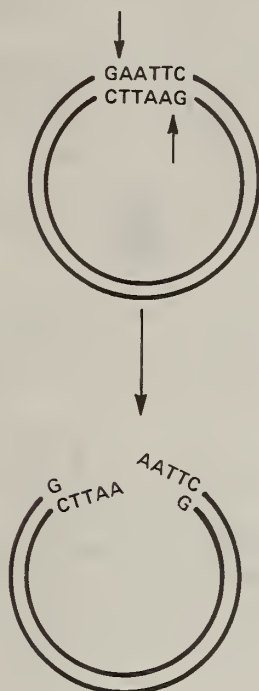
This method has several advantages, and is in fact being used in a number of laboratories to produce quite interesting types of molecular hybrids. Nevertheless, at the time it was reported, it was not widely adopted because it requires a number of special enzymes that are scarce, and also skills that are not widely practiced in many laboratories.

As so often happens, however, a simpler procedure to accomplish the same and more was found. Several years ago, Dr. Herbert Boyer and his colleagues at the University of California Medical Center in San Francisco isolated an enzyme that can break the DNA into small bits. These pieces vary in length, but they are large enough to contain from one to ten genes. Two Stanford biochemistry colleagues, Janet Mertz and Ronald Davis, established that the enzyme breaks the DNA in a special way. This was a unique finding because the break leaves very characteristic sticky ends on these bits of DNA.

May I have the next slide (3), please?

Slide 3

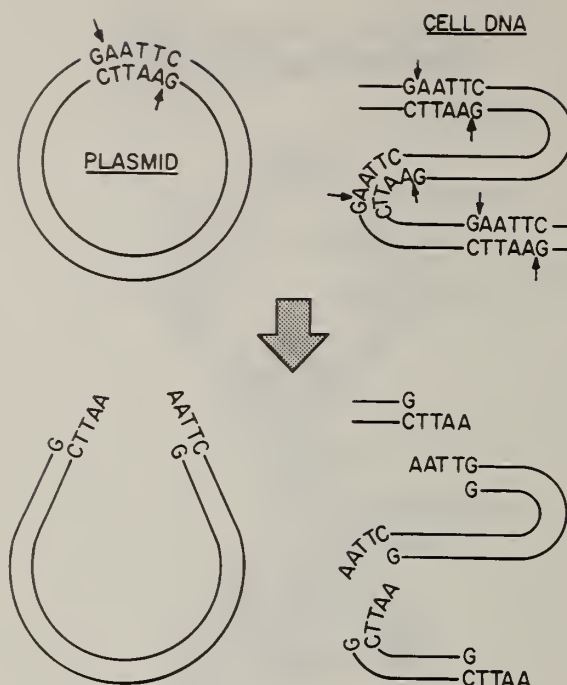
CLEAVAGE OF DNA BY R I
RESTRICTION ENDONUCLEASE



Just to show you, this is a molecule of DNA, and I focus only on a short sequence, six nucleotides out of the 5,000; and a particular enzyme, which as I said has been described by Herbert Boyer, was found to cut the DNA at the positions indicated by the arrows. So it cuts one strand between G and A, and a second strand here between the A and G, to produce a linear structure which has the short, cohesive or sticky ends, this end being able to pair or react with this end. It is possible to go from this state to that state very simply.

May I have the next slide (4), please?

To illustrate how the use of that enzyme has made it possible to join DNA molecules from different sources, here we have the DNA molecules indicated as such. Again, the position where this enzyme breaks, indicated by the arrow, is to produce two DNA molecules having the short, cohesive or sticky tails.

Slide 4

It is quite clear that one can take a molecule which has been cut by that enzyme which has the cohesive ends and take a completely different DNA from a completely different source which has been cut by the same enzyme. Because this enzyme leaves such cohesive ends on any molecule it cuts, these two molecules can be readily joined to each other through the interaction of these cohesive or sticky ends. One is therefore able to produce hybrid or recombinant DNA molecules which contain part of one structure and part of another structure, joined together by these chemical bonds.

Now, the endonuclease, or the enzyme which was discovered that carried out this type of cleavage, making it possible to join DNA molecules, was only the first of a family of enzymes capable of carrying out such reactions. Now we know that there are many others that can do the same thing, and there is no doubt that the list continues to grow as the months go by.

This was the first breakthrough, the development of a simple procedure for joining together DNA segments from very different sources. Ordinarily, when one carries out such joinings, one produces very complex mixtures of DNA segments and hybrids, often unresolvable, and therefore useless for experimental purposes.

Therefore, one is confronted with the question of obtaining a single pure type of recombinant DNA and this proposed a second challenge. In fact, it required a second breakthrough.

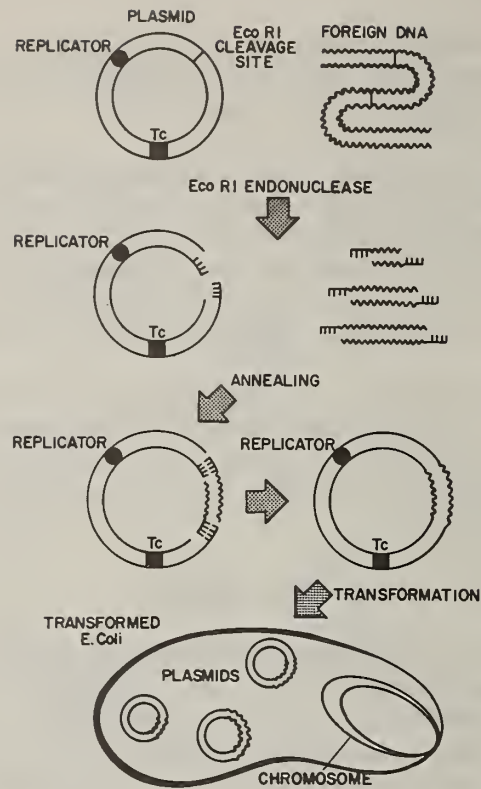
It was known that some bacteria contain plasmids. Plasmids are small, extra chromosomes that occur in cells in addition to their major chromosomes. A plasmid is also a piece of DNA, and it exists and propagates as a circular molecule. Such plasmids can be readily isolated in pure form and then reintroduced into other bacteria, where they can multiply autonomously -- that is, independent of the cellular chromosome.

A plasmid which turned out to be very useful was one carrying genes that confer resistance to the antibiotic tetracycline. Thus, the entry and the establishment of such a plasmid in a bacterium like E. coli can be monitored and, in fact, selected for by the use of tetracycline resistance for the modified bacteria.

May I have the next slide (5), please?

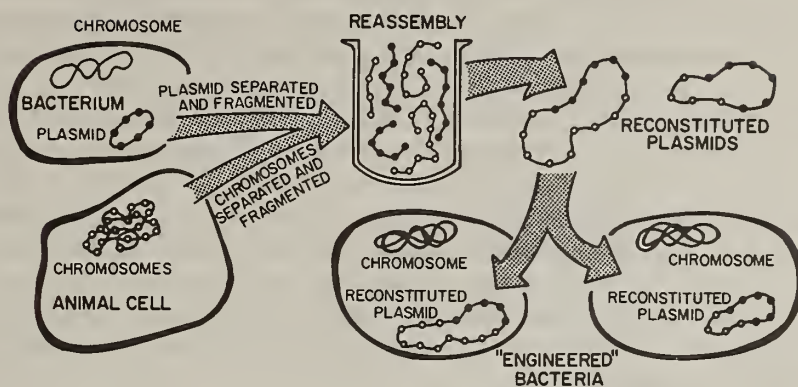
This is a strategy which was developed by Stanley Cohen at Stanford, and it involves the following. It is to obtain such a plasmid, and this is a particular plasmid which he used. That plasmid contains a sequence which can be cut by the enzyme I mentioned a moment ago, to produce a linear structure with short cohesive ends. It is then possible to take foreign DNA, and by foreign DNA I mean DNA from any source, any type of cell on this earth, cut that DNA with the same enzyme to produce short bits which have the same type of cohesive ends. It is only necessary to mix these two to produce a hybrid structure in which the ends of the plasmid are bridged by the segment of foreign DNA.

What Stanley Cohen showed, which was most important, was that these fused elements could be reintroduced into virgin bacteria where they can take up residence and multiply as the cell divides. The modified cells can grow on a very simple solution containing some sugar, a source of nitrogen, and a few minerals. But because they can divide roughly every 20 minutes, within a day one can obtain billions of organisms carrying the new DNA segment that they acquired, and this can be produced in pure form in large quantities. Therefore, it is a very simple matter to obtain segments of any DNA in large quantities in pure form.

Slide 5

The London Sunday Times had an elegant diagram, which is in the next slide (6), just to illustrate for a moment and to summarize this type of procedure. Here is a cell with its chromosome. This is the bacterium. We can obtain the plasmid, fragment it, and put it in the test tube. We can take the chromosomes of any animal, plant, or other bacterial cell, isolate its DNA and fragment it with the same enzyme and add it to the same test tube. They will re-associate spontaneously to produce a myriad of hybrid structures. One need only introduce these into a new bacterium in order to have them multiplied and purified, or what we refer to as being cloned.

One can then grow these bacteria literally in hundreds of gallon quantities to obtain very large amounts of this highly purified segment of the DNA of the original chromosome.

Slide 6GENETIC ENGINEERING WITH BACTERIAL PLASMIDS

I brought along a little set of beads to try to illustrate to you who would prefer to see it in this way. This is a set of beads, and if your imagination can be allowed to project it to be a double-stranded DNA molecule, the segments here are meant to indicate different genes located linearly along the DNA molecule.

What was found was essentially that there is an enzyme available that can cut the DNA at specific locations to produce bits. So we can produce a large number of different kinds of segments, each of them derived from a specific region of the cellular chromosome.

Now, these bits can be joined together in any random fashion to produce new arrangements by just joining the ends. I don't know what the order was that I had before, but it is clearly different now from what I showed you a moment ago. But that isn't the useful technique. The useful technique is to be able to obtain these bits in highly purified form, and for that there is our friend, the plasmid.

This circular structure is meant to indicate the nature of a circular DNA which can serve as the vector for carrying segments of DNA and for amplifying them through bacteria. What I said needs to be done is to break the plasmid in such a way as to convert it into a linear structure, and

then take whatever gene you want to purify, mix it with that plasmid, and cause the re-association of these two structures to reform circular molecules. It is only necessary then to re-introduce these into appropriate bacterial cells in order to create or propagate this new chromosome, and specifically the segment which was derived from the complex chromosome which is now in a simple structure which we can isolate and prepare in large quantities.

Another attractive and potentially very useful approach to the isolation and amplification of genes has been pioneered by Noreen and Kenneth Murray in Edinburgh, and by Ron Davis at Stanford. Using the same methodology that I have just described, they can incorporate DNA segments into the chromosome of a small bacterial virus. In this way the new genes behave and multiply as if they have an integral part of the viral chromosome.

Cloning or obtaining a pure preparation of a single chromosomal DNA segment is not restricted to bacterial vectors and cells. Some animal viruses are analogous to bacterial plasmids in their structure, and because they can multiply in cultured animal cells, they can in fact be used. They can be joined to foreign DNA segments, and the hybrids can be propagated in animal or human cells.

Now, why do biochemists want to do these kinds of experiments? There are many important, fundamental, and practical questions that could be answered or approached by this type of research. We need to understand the structure of genes and how they work, and this methodology provides us with a simple, inexpensive, and elegant way to prepare large quantities of specific genetic bits of information in pure form. There is little doubt in my mind that our inability to achieve this earlier is what slowed down progress in understanding the genetic chemistry of cells of higher organisms.

In the short space of only a year, Hogness and his colleagues at Stanford, as well as Don Brown here in Baltimore, have shown how powerful a tool the recombinant DNA methodology can be for the isolation of genes and the analysis of chromosome structure. The precise chromosomal locations of discrete segments of DNA from the fruit fly Drosophila have been mapped, and that information promises new and important insight into the perplexing problem of how genes are organized into complex chromosomes.

Understanding how the genes of higher organisms are expressed and regulated, which is a pious dream we had a few years ago, is literally now within our grasp.

So far I have emphasized only the ability to clone and amplify foreign genes in bacteria. But there is another and even more promising dimension. If the genes, for example, of toads, flies, or humans function in a simple bacterium, then we can certainly begin to study the expression and function

of these genes in an organism that is extremely well understood, perhaps the best understood organism on our planet [Escherichia coli].

We might also learn how defects in the structure of such genes alter their function and their regulation. But there is still some question as to whether the genes of higher organisms can be expressed after their introduction into E. coli. It is quite clear that the genes from bacteria, any bacterium, are likely to be expressed in E. coli., but there is still some question about whether the genes of animal cells or plant cells can in fact be normally expressed in this simple organism.

Is this a fundamental or a technical obstacle? The answer is that we don't know at the moment. Whether or not this obstacle can be surmounted, I believe has a very important bearing on the question of evaluating both the potential benefits and the risks that accompany these experiments. I may have a chance to come back to that later.

Although there hasn't yet been a practical application of the new techniques, there is every reason to believe that they will in fact have a very significant impact on medicine, industry, and agriculture. This methodology offers us the prospect of isolating genes that code for pharmacologically important substances. Specifically, the genes for insulin, growth hormone, or immunoglobulins could be isolated and propagated in a simple bacterium such as E. coli. And if, as I said before, these foreign genes were expressed in the bacterium, then these organisms could well become factories for producing some of society's most needed supplies.

The production of antibiotics and vitamins could be revolutionized by eliminating the need to grow and process exotic bacterial and fungal strains or to use specialized tissues as a source for these precious agents. Both might be synthesized by E. coli in a simple medium.

There are also important potential benefits to expanding the world food supply. The availability of natural and artificial fertilizers limits certain crop yields. But atmospheric nitrogen is an infinite source of ammonia, if only we could harness the microbial potential for nitrogen fixation. Experts in this field suggest that the introduction of the nitrogenase system from bacteria into plants or into symbiotic organisms is imminent and promises great rewards.

It is also conceivable that crop production could be increased by introducing genes which increase the efficiency of photosynthesis, or which enhance the nutritive value of plant products.

I have no doubt that in time the opportunities will expand as the methodology becomes more sophisticated. The isolation of genes puts us at the threshold of a new form of medicine, with the treatment of crippling genetic diseases through the replacement of defective genes by their normal

counterparts. Though this approach is simple and attractive in principle, this step has many pitfalls and unknowns, and these shall certainly have to be examined very carefully before any such therapy could be considered.

I have spoken about the benefits briefly. There are many others, and we could spend hours discussing them. I want to turn now to the question of risks.

From the beginning, some people expressed reservations about the risks attending the construction and propagation of hybrid or recombinant DNA molecules. In fact, I had been the target of that concern, nearly 4 years ago, when we had contemplated putting tumor virus genes into E. coli. But a more concerted action occurred when scientists attending the 1973 Gordon Research Conference on Nucleic Acids requested that the National Academy of Sciences and the Institute of Medicine consider the question of whether this research created potential hazards to man and his environment.

Subsequently, at the request of the Academy, I convened a group of about a dozen scientists to meet during April of 1974 at the Massachusetts Institute of Technology to consider this question.

Despite our own strong commitment to this work, we felt it necessary to call attention to the potential -- and here I would like to stress the word potential, because none of the risks that I have talked about can be documented as having occurred or being very likely to occur. They are potential risks.

Nevertheless, we felt it necessary to call attention to these potential risks of several lines of work with biochemically constructed hybrid DNAs. We suggested that scientists working in this field throughout the world should join us, the signators to the letter or report which was published in Science and Nature and the Proceedings of the National Academy of Sciences, should join us in deferring, and I stress the word deferring, voluntarily, at least two kinds of experiments. This pause was to be a temporary measure to secure time until an international meeting of consultants with greater expertise and broader concerns could evaluate the nature and the magnitude of the potential risks.

Though each individual assesses the risks involved with the construction and propagation of recombinant DNAs differently, there are few, if any, that I know of who believe that this methodology is free of any risks. What then, are the possible risks?

First, there is the possibility of genetically altering bacteria so that they have a greater capacity to cause disease or to resist treatment. Genetic determinants promoting pathogenicity are known to occur on bacterial plasmids that are being used to introduce and transfer genes into bacteria. Particularly risky is the possibility that certain infections

treatable with present-day antibiotics could be made resistant to that therapy. The introduction of genetic determinants conferring antibiotic resistance or combinations of antibiotic resistance into bacterial species not now known to contain them could compromise the effectiveness of some of our weapons against infection.

For example, the beta hemolytic Streptococci and certain Pneumococci bacteria which can produce serious infections in man, are even today generally killed by relatively low levels of penicillin. The inadvertent or even the intentional introduction of genes conferring high levels of penicillin resistance to these organisms would be most unfortunate if such a strain got loose in the population.

Similarly, the transformation of relatively harmless bacteria into ones that can produce toxins such as diphtheria, cholera, or other enterotoxins would certainly seem to constitute a significant risk.

An additional concern is that fragments of foreign DNA introduced into bacteria may include, in addition to the genes which the investigator has selected for study, other genes which he has not yet identified and which, if they became established in natural E. coli populations, might release harmful products for man, plants, or animals.

Another risky experiment is to introduce genes from tumor viruses into bacteria. In fact, it was just such an experiment that my own laboratory had proposed and deferred more than 3 years ago. SV40, adenovirus, and herpes virus can cause cancer in animals. We could learn something about how this occurs if we could put all or parts of these viral chromosomes into a simple bacterium like E. coli. But our intestines are full of E. coli, and one may ask whether it would be harmful to have E. coli in our gut carrying genes from viruses that can change normal cells into cancer cells.

The answer to that question is that we don't know, and moreover, the answer is very difficult to obtain. It is possible that such infections might not produce immediate symptoms, and therefore it might be years before harmful effects of a tumor virus became evident.

One very powerful feature of this technology is the ability to clone specific DNA fragments from the total DNA of plant and animal cells of diverse sources. We refer to such experiments as "shotgun" experiments. Inasmuch as many mammalian and even lower animal-cell chromosomes are known to contain sequences common to tumor viruses, the indiscriminate and careless handling of modified bacteria produced in such experiments could loose carriers of carcinogenic potential.

A particularly worrisome feature of E. coli is its untiring promiscuity. In fact, it is that very attribute that has made E. coli a favorite

bacterium of the molecular biologist and the geneticist for the last 25 years. E. coli can mate with a variety of organisms it comes in contact with, both to donate and receive new genetic material. Moreover, it readily transfers the small plasmids to other cells. These plasmids could then become widely disseminated among other bacteria that inhabit animals, humans, and the soil.

So much for the risks. Let me now turn to the response to that National Academy of Sciences report which was published in 1973.

How did the scientific community respond to that unorthodox call for a pause in what was clearly a particularly exciting line of research? To my knowledge, during the time the appeal was in force, there was no explicit violation. I have no evidence nor have I heard of any which suggests that scientists were stealthily carrying out proscribed experiments in the dead of night or on weekends. The Directors of the National Institutes of Health and the National Science Foundation made available funds to convene the international conference which was to consider appropriate ways to deal with the postulated hazards. The NIH also appointed a National Advisory Committee to consider these issues. Official bodies from Great Britain, France, Germany, Holland, the Soviet Union, Australia, Japan, all met to consider their response to the mounting pressures to proceed with this work in their own countries.

I believe the most important outcome of that report was the debate and response among the scientific community itself. Discussions of hazards and safety began to be considered, together with the scientific protocol for likely experiments.

In February, just a year ago, the International Conference on Recombinant DNA Molecules was held at Asilomar, Pacific Grove, to discuss appropriate ways to deal with the potential biohazards of this research. The conference participants, which included besides scientists, lawyers, and representatives from government and industry, recognized that they were not a legislative body, and therefore they were not empowered to license or to approve experimental protocol. The group had absolutely no policy-making function or directive. Our mandate was to provide evidence and advice--advice concerning the best available estimate of the risks associated with the recombinant DNA methodology, and advice as to what action could be taken to deal with such risks.

Without any power the only thing we had available was the reasonableness of our arguments, and moral suasion. Only those could influence our colleagues elsewhere in the various bodies that were entrusted with making policy.

The key concern of the meeting was whether the work should continue, or could continue, with minimal risks to workers in the laboratory, to the public at large, and to the plant and animal systems sharing our planet.

After extensive discussions and debate, heated most often, the participants at the conference agreed that most of the work on the construction of recombinant DNA molecules should proceed, provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, were used.

Certain experiments, however, though they were beyond criticism on scientific grounds, were believed to pose hazards of such a magnitude that they should not be carried out at the present time. Conference participants adopted the principle that containment was an integral part of the experimental strategy, and that the stringency and effectiveness of the containment should match the potential risks. It is quite clear that estimating the risks is difficult, and in the beginning very likely to be intuitive, but there is great hope that our ability to estimate such risks will improve as we acquire additional knowledge.

It was accepted as a principle and as a desirable one to set the containment requirements high at the beginning, and then to relax them as experience permitted. Now, the containment of potentially biohazardous agents can be achieved in several ways. The most important one, I believe, because it contributes most significantly to limiting the spread of any of the organisms carrying recombinant DNAs, is the use of what came to be known as biological containment. Biological containment can be achieved if (1) the plasmid or viral DNA into which the foreign DNA has been inserted is able to propagate only in specified bacterial hosts, and only under defined conditions and (2) the bacterial hosts are unable to survive outside the laboratory environment.

To me, one of the most refreshing and exciting outcomes of the Asilomar exercise was the way the genius of today's molecular geneticists took to the task of constructing what one reporter called fail-safe organisms.

Now, physical containment, exemplified by suitable hoods or special rooms which have limited access or negative pressure was recommended as an additional factor of safety. In addition, the adherence to good microbiological practices, which can limit the escape of organisms from the experimental situation, is essential to the safety of the operation. Consequently, the education and training of all personnel involved in these experiments is mandatory. We agreed that in practice these different means of containment would complement each other, and substantial improvements in the ability to restrict the growth of bacterial hosts.

I would like to make just a few final comments, and that is to emphasize something that Dr. Fredrickson pointed out. The concern about the potential risks of this type of work was in fact first perceived and raised by the scientists, particularly those who were working in the field. Some have commented on whether this was a unique social action. I care not to comment on that, but I believe that what has followed since reflects the

awareness and the sense of responsibility of the bulk of today's practicing scientists.

The initial report to the National Academy of Sciences and the follow-up report of the views of the Asilomar conference were intended to provide informed opinion as to the risks of such research, and advice on the most reasonable course of action. Everything took place in the open, with extensive public overview. That advice has now been reviewed and its principles accepted in the guidelines which have been developed by the NIH committee, or I should say the national committee, and have been used as the basis of recommendations that are now being considered by the Director and at this meeting.

I believe that they represent a responsible and prudent first step to dealing with this problem. I stress first step because only further experimentation accompanied by vigilance will tell us how to proceed to the next step.

My own personal view is that the guidelines are stricter than they need be on the basis of scientific evidence alone, but I and many of the scientific community are prepared to accept them as the price for exploiting this powerful new tool of biomedical research.

Thank you.

DR. FREDRICKSON: Thank you very much, Dr. Berg. In keeping with my policy stated earlier that we would like to get these scientific details out for the record, before we have any questioning, I am going to suggest that we proceed directly, at this point, to a brief report from Dr. Stetten concerning the developments after Asilomar, with particular reference to the NIH committee.

I will again remind you that you will have opportunity to discuss with Dr. Berg this presentation at the appropriate time, later in the day.

DR. STETTEN: In response to the letter alluded to, signed by Dr. Berg and others, in October of 1974 a standing committee of the National Institutes of Health was authorized and I was invited to be its chairman.

The first meeting of this committee occurred on the day after the Asilomar conference. It has met four times in all, first in February of 1975. Again in May, it met in Bethesda. In July it met at Woods Hole, and finally, in December it met at La Jolla. The committee, originally authorized at a strength of 12, has since been expanded to a strength of 16. There are 14 members at this time.

The charge to the committee was to advise the Director of NIH on the conditions which should be imposed upon grantees and contractors of the NIH before they be funded to conduct research in the area of recombinant DNA molecules. It furthermore was given a charge to explore needs, national needs, for the construction of high-containment physical facilities insofar as these might be indicated, and in addition it was charged to give such support as may be needed to the increase in the safety of the handling of these interesting and important materials.

The membership of the committee you will find in your brown notebooks under Tab E, page 3, I believe. I am sorry, page 5. The membership as printed is incomplete. We are happy to add one member who is here today, Dr. Emmett Redford.

The committee itself early resolved that we should have at least one member who is not from the biomedical science domain, and we were very happy therefore to be able to recruit Dr. Redford, a Professor of Political Science at the University of Texas, Austin, who has a long and distinguished career of public service and teaching.

Dr. Redford attended our last meeting as a consultant, and is now a full-fledged member of our committee.

In addition to the meetings of our committee, there has been considerable interest in this area, as evidenced by hearings in Senator Kennedy's committee on April 22, 1975, at which time some four scientists--Dr. Gaylin, Dr. Cohen, whose name was mentioned earlier, Dr. Brown, and Dr. Holman--testified before the committee on their views of the import and the hazards of this line of research.

I would like now briefly to summarize what I regard as some of the accomplishments of the committee apart from its deliberations on the guidelines, of which you will hear more. It was early recognized that it would be very important to disseminate promptly among workers in the field any significant advances which may be made, either in the perception of hazards or in the development of safeguards.

In view of the delays in traditional journal publication, it was therefore determined to establish a newsletter. This responsibility was assumed by the National Institute of Allergy and Infectious Diseases. Dr. Chamberlayne of that Institute put together a newsletter based on his earlier experience with similar newsletters in other rapidly developing fields of science.

Dr. Jacobs has some sample copies of this newsletter. The first issue came out in September. The second issue also has come out. It is projected to appear four times a year as a minimum. Any reasonable applicant will receive copies of the newsletter, but a condition has been

imposed for the time being, anyhow: that all beneficiaries of this newsletter will be expected to make at least one contribution to it each year. Promptness of publication will be its chief goal. It will not be, I think, a place for the publication of new basic science, but rather of new technology directed toward increasing safety.

It was determined to ascertain what higher level of physical containment already existed in the United States. Our local expert, Dr. Emmett Barkley, from whom you will hear later, was given this responsibility, and he has identified some 27 facilities in the United States which are well designed to handle noxious microbial agents. Some nine more apparently are under construction. These are variously Federal, state, or university facilities. They have been solicited to ascertain their availability for other scientists who may need such facilities for the conduct of, let us say, an occasional experiment.

Two such facilities are under the direct control of the National Institutes of Health. One is our own Building 41; the other is at the Frederick Research Center, some 30 miles from here, where high-containment facilities already exist.

An area of importance where it was determined by the committee that research should be stimulated was the development and testing of safer microorganisms. Five distinct requests for proposal were drafted. These were for the design or the construction of safer bacterial hosts, of safer plasmids, of safer bacteriophages, for the design of tests to measure the survival of these and other forms, and for the design of tests to study the genetics of these forms.

I can report to you that we have received proposals in all five fields. One proposal in one of the fields, and more than one proposal in the other fields. Review committees have been named which will study these proposals, and it is everybody's expectation that contracts in these areas will be awarded within the near future.

It was deemed desirable that we establish stock centers for microorganisms. Since there was already a stock center for Escherichia coli, the microorganism which Dr. Berg mentioned earlier, at Yale University, it was not hard to persuade Dr. Adelberg of that university to extend the stock center's responsibility to include such mutant forms of E. coli as may be developed in the quest for a safer, or what may be called a self-destructing organism.

Any strain of organisms deposited in such a stock center would of course be available to any responsible investigator who required such an organism.

In addition, it was agreed by Dr. Szybalski of the committee at the University of Wisconsin, to provide a similar service for bacteriophages as these may be developed in relation to this program.

It was widely felt by members of the committee that there were people working in this field who had in their education somehow or other avoided taking a course in medical bacteriology, and who therefore might not be aware of the practices which are dictated by good bacteriological technique to prevent unnecessary spills and accidents.

The National Cancer Institute has, in the past, provided short courses at various centers throughout the country which at that time were directed toward people who worked with cancer-producing, so-called oncogenic viruses. It was agreed by Dr. Barkley that based on this experience, additional courses would be made available to people working in the field of recombinant molecules so that they might avail themselves of necessary bacteriological technique.

The committee also directed that certain studies be made by members and reported to the committee. The first of these, which unfortunately by oversight was not included in your brown books, was a study by Dr. Stanley Falkow of the University of Washington, Seattle, on the ecology of E. coli. Since it was clear from all early deliberations that this was a favored organism for this kind of study, it became important to collect in one place a substantial fraction of all that is known about the relationship of this organism to its environment, which includes Homo sapiens. Such a study was completed, and has been distributed.

In addition, a second contender, which will be mentioned later on, I am sure, for the position of a favored bacterial host was Bacillus subtilis, an organism which has no intimate ecological contact with man, and therefore was considered to be perhaps less hazardous, only unfortunately, less well studied.

The committee has supplies us with a report on the present state of knowledge of Bacillus subtilis, which you will find under Tab G in your brown books, as Appendix A.

In addition, a study was made by Dr. Rowe of the National Institute of Allergy and Infectious Diseases and a member of our committee, of the uses of the polyoma virus, this being a mammalian virus which seemed to be the most likely contender for the honor of being first incorporated into the DNA of some other microorganism.

In addition to these papers, a meeting was sponsored by a member of our committee, Dr. Helinski, which immediately preceded the La Jolla December 4th and 5th meeting of our committee. The name of this workshop was Design and Testing of Safer Prokaryote Vehicles and Bacterial Hosts for

Research on Recombinant DNA Molecules. I have not worked out the acronym for that meeting.

The meeting was generally held to be greatly or enormously successful, and some of that which was reported will certainly be discussed here, I would hope, by Dr. Roy Curtiss, when he has this podium. Indeed, the effect of the science which was announced at that meeting was quite demonstrable upon the attitudes of the committee meeting which immediately succeeded it.

All of our meetings have been advertised and public. They have all been open, with the exception of one brief period of time during which it was our assignment to review grant applications. It is still traditional and approved, apparently, to hold review of grant applications in a confidential mode, since the application itself, at least until it is funded, is held to be confidential. But with that brief exception of a half hour, the meetings were public. I believe press was present at all of the meetings, and the meetings were reported extensively.

We had, in addition to the members, a number of liaison associates from various other agencies, and one whom I would like particularly to single out, Dr. Herman Lewis, who attended all of our meetings, representing the National Science Foundation, since it was apparent to all concerned that it would be desirable, whatever policy was reached, to have the policy of granting by the National Science Foundation and by the National Institutes of Health compatible with each other.

In some of the meetings, there was representation from foreign interests, particularly from Britain, and from the European Molecular Biology Organization, EMBO, which is centered at Heidelberg.

At our first organizational meeting it was recommended that, pro tempore, we accept the document which was yet to come out of the Asilomar conference as a guideline, and during the early months after the document was distributed, grants in limited number were awarded insofar as they conformed to the principles in that document.

At the present time, however, we have asked that all grant applications and fellowship applications relating to DNA recombinant molecules be referred to our committee. We have, by way of example, at the present moment nine such applications on the table which are currently being reviewed, and the results of these reviews will then be referred to the appropriate study sections, where these grants will be studied for the merit which they contain.

In addition to all of these activities, consideration has been given to the desirability that we institute here at the National Institutes of

Health certain experiments which will be particularly informative with respect to the general hazards of the procedure. Thought has been given to such experiments. Dr. Wallace Rowe and his staff have been giving a great deal of attention to this problem, and it is not unlikely that in the reasonably near future such experiments will be undertaken, probably here in our own high-containment facility.

Now a word about the guidelines. At our first meeting there was rather wild discord among the participants. I came home personally despairing that that we would ever come out with anything that would be useful. It has been a very interesting experience to me to see this committee take hold of a problem and finally behave as a committee and produce a document.

At our second meeting it was determined that we could not draft the document in congress, and we therefore asked one of the members who had much experience in this business, Dr. David Hogness of Stanford, to chair a committee, a subcommittee of four which would prepare a draft for discussion. Dr. Hogness did just this and produced a fine, workable draft in consultation not only with the other members of this committee, but I believe, with Dr. Berg and with many other associates in the field.

This was the document which was reviewed subsequently at Woods Hole in July, and at that point every clause in the document was discussed and was voted upon, and a second draft, which was called the Woods Hole draft was reached. This was distributed informally and elicited a rather massive mail response. I would guess on the order of 50 letters came to my office within the succeeding few weeks. The initial crop of letters quite uniformly complained that the Woods Hole document did not impose adequate constraints. Curiously, as the weeks went by, the tone of the letters changed, and the later letters were almost as emphatic and of the opposite design. They were complaints that we had been much too stringent in our recommendations.

It was clear that we were dealing with a situation in which there were many diverse opinions, and after consultation with Dr. Jacobs, Dr. Gartland, Dr. Talbot, Dr. Maxine Singer, and others concerned, it was determined that we had better have another go at it.

Another member of the committee, Dr. Elizabeth Kutter was asked if she would prepare a revision of the document, and a third draft ultimately came out as a result of Dr. Kutter's efforts and numerous consultations with various scientists in the field. Before we went out to La Jolla, it was clear that we had a fairly chaotic situation with three separate and distinct drafts, and in order to accommodate to this Dr. Talbot undertook the heroic job of generating what I have called the Variorum Edition. Those of you who know Furness's Variorum Edition of Shakespeare will know what I am alluding to.

This was a massive document. It had three columns. The first column was the entire text of the Hogness draft. The second column was devoted to the Woods Hole draft, and the third column was devoted to the Cutter draft, which showed every single variant reading. This included spellings, punctuation points, underlinings, bibliographic citations, everything. It also included in some instances fairly massive deletions, fairly massive insertions, and some fairly extensive textual revisions. There were, in all, as one looked through the document, about 250 points of variation. Many of these were trivial; I was happy when the committee agreed to pass to me the responsibility of deciding whether it should be a semicolon or a colon.

On the other hand, many of them were quite substantive in nature. These were discussed at La Jolla in great detail.

On each issue a vote was taken, and the vote of the majority was accepted. On some issues none of the three variants appeared to be acceptable to the committee, and new drafting was done. The committee labored far into the night on two successive days, and the guidelines which are before you represent the result of this effort. By the time the meetings were over, the committee was truly functioning as one might wish a committee to function, with a commonality of purpose and with recognition that there was a serious job to be done and, I think, some satisfaction that perhaps they had approximately done it.

It is my belief, and I think that of the majority of the committee--and Dr. Berg, I think, would agree--that there is no final guideline. Even in these guidelines we have detected certain inconsistencies which will have to be eliminated. When this is done, new experimental data will continue to come in which will cause us, from time to time, to modify our estimate of hazard and the constraints imposed. Therefore this is a standing committee. Its next meeting is scheduled for April 1st and 2nd. I would imagine it would meet three or four times every year for the next several years to review what it has written and what needs to be changed.

Thank you very much.

DR. FREDRICKSON: Thank you, Dr. Stetten.

No amount of genetic research will ever, I suppose, eliminate the next phase of the agenda. But before you go, I would like to remind you of two things. One is that I have reminded you earlier of the materials already sent to the committee. There are copies of some, particularly some recent submissions from the Boston Area Recombinant DNA Group and the Genetics and Society Policy Group, which are reproduced on the table behind you for those who wish them.

Now, I would remind you that the chair will reconvene this meeting at precisely 10:50, at which time Dr. Singer will begin. We now adjourn for coffee.

(Brief recess.)

DR. FREDRICKSON: I would like to announce that some of the material that I advertised as being available has now been completely consumed. More copies are being made and will be available after lunch.

We will resume now our session with a presentation by Dr. Maxine Singer, who is from the Laboratory of Biochemistry of the National Cancer Institute, a molecular biologist, but I believe not immediately working on recombinant DNA experiments herself at this present time. Dr. Singer will perform the difficult task of providing us with a summary and review of the proposed guidelines.

Maxine?

DR. SINGER: The guidelines that I am going to try to summarize for you this morning are the guidelines discussed and voted upon at the meeting of the advisory committee in La Jolla on December 4th and 5th. I am going to try to present a detailed view of the guidelines, but because of the time limitation, it will not be a comprehensive view.

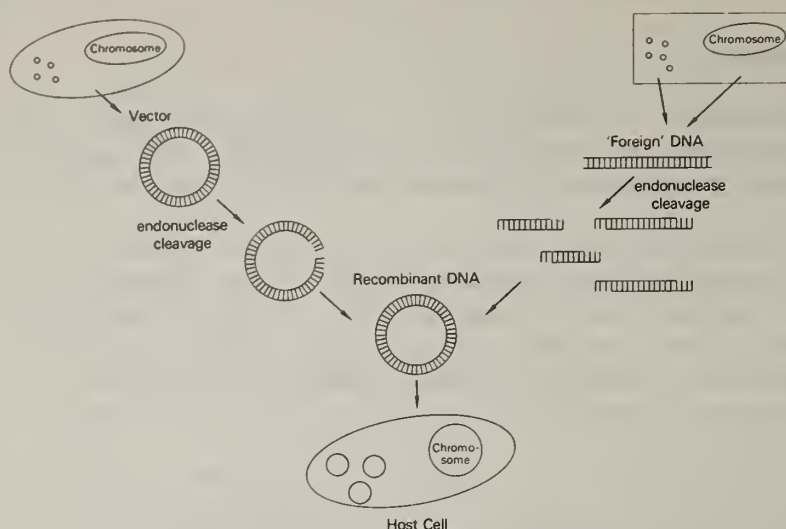
Dr. Berg has already defined the kinds of experiments to which the guidelines apply, and the first slide that I want to show you summarizes it again, and will serve to define a few of the terms as they are specifically used in the guidelines.

Could I have the first slide, please? It is very similar to a slide that Dr. Berg showed you.

At the upper left is a cell containing chromosomal DNA and several small, independent genetic elements. These small, independent DNA molecules can be isolated from the cell and can be manipulated to serve as one portion of the recombined DNA, the portion which is called the vector.

Most such elements are circular DNA molecules, and they can be cleaved, as shown, by restriction endonucleases, to yield linear DNA strands that have either sticky ends or ends that can be made sticky.

In most experiments the recombinant DNA will finally be reinserted into cells of the same species from which the vector was isolated, and it is the genetic information encoded in the DNA of the vector which ultimately is responsible for the continued existence and replication of the recombined DNA in the recipient cell.

Slide 1

At the upper right of the slide is another cell, shown here as a rectangle. This cell will serve as the source of the DNA to be joined to the vector. The DNA is termed the foreign DNA, and it too can be cleaved by restriction endonucleases. The rectangular cell can represent a cell from any living species, and as shown here the foreign DNA might contain chromosomal DNA or independent genetic elements, or both.

The foreign DNA fragments are, in the next step, joined to the vector by procedures that were outlined by Dr. Berg, and the recombinant DNA is then reinserted into a host cell. Again, the host cell will most likely be of the same species as used for the isolation of the vector.

The cells are then placed under conditions where they grow and multiply by division, and the resident recombinant DNA will be duplicated along with the chromosomal DNA, and each new cell will contain recombinant DNA.

Now, in the experiments discussed in the guidelines, the host cells are generally single living cells, either microorganisms such as bacteria, or animal or plant cells which were originally obtained from living tissue, but are grown as single cells under special laboratory conditions.

May I have the lights please, and not the next slide yet.

The proposed guidelines start with a statement of general principles, and these are consistent with the general conclusions that were published in the report of the international conference at Asilomar, and were reviewed

earlier by Dr. Berg. The first principle, as stated in the guidelines, is that there are certain experiments which in the light of currently available information may be judged to present potential hazards of so serious a nature that they should not be attempted at this time.

The second principle is that there is a large group of feasible experiments which appear to pose lesser or no potential hazard, and therefore can be performed, provided that the information to be obtained or the practical benefits anticipated cannot be obtained by conventional methods, and provided that appropriate safeguards for containment of potentially hazardous organisms are incorporated into the design and execution of the experiment.

Third, that the more serious the nature of any possible hazardous event, the more stringent should be the safeguards against escape of the potentially hazardous agents. Since the estimation of potential hazards is currently conjectural and speculative, the levels of required containment for potentially hazardous organisms should be set high initially, and modified only when there is substantial relevant information to advise such modifications.

The fourth and final principle is that the guidelines are to be reviewed at least annually, in order to account for new information.

There are three approaches to the problem of containing potentially hazardous organisms which form the basis of the safeguards recommended by the guidelines. Each of the three may be viewed as setting up barriers to the dissemination of potentially hazardous organisms from the laboratory situation, and as setting up barriers between the laboratory worker and the organisms. Two of these approaches involve the limitation of the actual physical escape of the organisms and are referred to as physical containment.

The first such approach is the set of standard microbiological practices that have been developed over a period of many years and are widely used for handling pathogenic organisms both in research and clinical laboratories. In the hands of well-trained personnel, these procedures are proven to be effective in safeguarding both the worker and the environment from the spread of pathogenic agents.

The second approach to physical containment involves the use of special kinds of equipment and facilities to limit the spread of aerosols and for decontamination and for containment of laboratory air and wastes, as well as limitation of access to laboratories.

As with the standard microbiological techniques, these types of equipment and facilities are not new, but have been developed and used previously for containment of known pathogenic organisms. The guidelines go into some detail concerning the practices and facilities required for physical

containment. Later today Dr. Barkley will describe some of the details for you, and also show a movie which describes the strictest available facilities.

For now, it will be sufficient for me to tell you that the guidelines specify four levels of physical containment. They are termed P1, P2, P3 and P4 in the document, in the order of increasing levels of containment.

P1, the lowest level, consists of the use of the standard microbiological practices that I mentioned before. The P2 and the next higher level, P3, each require special procedures and facilities designed to limit to increasing extents any possible accidental escape of potentially hazardous organisms.

Finally, P4, the maximum level of containment requires sophisticated and isolated facilities designed for maximum containment. Each of the levels P2 through P4 assumes that the techniques demanded by P1, the standard microbiological practices, will be followed; and furthermore, for each level, the training of personnel in the relevant procedures is mandatory. Training is to include both the technical manipulation and instruction in the biology of the relevant organisms and systems.

The third approach to the problem of containing potentially hazardous organisms within the laboratory is termed biological containment. Biological containment is defined as the use of host cells and vectors with limited ability to survive outside the very special and fastidious conditions which can be maintained in the laboratory, but are unlikely to be encountered by escaped organisms in natural environments. Biological containment is an integral part of the experimental design, since the host and vector will need to be chosen in any given experiment with a view both to the purpose of the experiment and to containment. It is important to stress, as the guidelines do, that physical and biological containment procedures are complementary to one another, each one serving to control possible failures in the other. The use of both in a given experiment affords much higher levels of containment than either one alone; therefore, as you will see, the guidelines always recommend both a particular level of physical containment and a level of biological containment for any given experiment.

It is also of some importance that the guidelines recommend that publications describing work on recombinant DNA include a description of the containment procedures that have been used.

The first class of experiments described in the guidelines are those which are not to be carried out at the present time. While it may be argued that a combination of P4 physical containment and a high level of biological containment could essentially contain these recombinants, the magnitude of the possible dangers, were containment to fail, dictates that these experiments be deferred. The class of experiments is summarized on the next slide.

May I have the next slide (2), please?

Slide 2

Experiments Not to be Initiated at Present

1. Experiments involving DNA from classes 3, 4, and 5 (CDC)
2. Deliberate formation of recombinants when genes for dangerous toxins are present.
3. Deliberate formation of recombinants from plant pathogens if likely to increase virulence or host range.
4. Widespread release into environment of any organism containing recombinant DNA, except when no reasonable doubt of safety.
5. Transfer of drug resistance traits to new microorganisms, if transfer could compromise drug use in medicine or agriculture
6. Large scale experiments if harmful products made. Exceptions possible with permission of Advisory Committee.

First, any experiments in which a portion of the recombinant DNA derives from pathogenic organisms listed under classes 3, 4, and 5 of a document entitled "Classification of Etiologic Agents on the Basis of Hazard," that is published by the Center for Disease Control--that is, the CDC, United States Public Health Service. This document categorizes naturally occurring organisms and viruses known to be pathogenic to man, and to agriculturally important species on a scale of increasing hazard going from 1 to 5. Class 5 agents are excluded from the United States by law. Class 4 includes such agents as wild-type smallpox virus, and wild-type yellow fever viruses. Class 3 includes such agents as arboviruses, which are responsible for encephalitis, psittacosis agents, rickettsial agents. Class 2 includes agents which may produce diseases of varying degrees of severity if accidentally inoculated into laboratory workers, but which are considered normally containable by standard laboratory practice. These include bacteria such as species of Salmonella, agents which cause amoebic dysentery, viruses for mumps, measles and rubella. Class 2 agents may be used in recombinant DNA experiments.

The second group of experiments involves the deliberate formation of recombinants containing the genes for toxins of very high toxicity. Examples of this group of toxins are the botulinus toxin or diphtheria toxin.

Third, deliberate formation of recombinant DNA using the DNA from plant pathogens if the resulting material might increase either the virulence of the pathogenic material or the range of species susceptible to the disease.

The fourth group of experiments which are not to be initiated at present are as follows: certain of the possible beneficial applications of DNA recombinant research involve the creation of organisms with the ability to carry out useful environmental functions. Release of such organisms into the environment may, at some point, be required to test their efficacy and certainly to make use of them. The guidelines advise that widespread release of any organism containing recombined DNA not be undertaken at present unless a series of controlled tests leave no reasonable doubt as to their safety.

Fifth, the resistance of various clinically important microorganisms to drugs, such as antibiotics is known to result from particular genes encoded in DNA. Recombinant experiments could therefore result in the introduction of resistance into species that do not naturally acquire such resistance. The guidelines advise that such experiments be deferred if they could compromise the use of a drug for the control of disease agents in medicine or in agriculture.

Finally, the guidelines advise that experiments with recombinant molecules be limited in scale to volumes less than 10 liters if harmful genes are present. This recommendation reflects the greater probability of escape of organisms when large volumes are manipulated.

The guidelines state that the advisory committee may make exceptions to this rule for particular experiments deemed to be of direct societal benefit.

May I have the lights, please?

The proposed guidelines then proceed with the classification of currently permissible experiments. Recognizing the relation between the host-vector system required by the experiment, and the design of suitable biological containment, experiments using the same host-vector system are grouped together in the document. At present the system of choice for many experiments is the common laboratory bacterium E. coli--in particular, strain K12--and independent genetic elements known to reside in this strain.

There are two factors contributing to this conclusion. The first is that the strain has been studied extensively and can be readily manipulated for recombinant DNA experiments. Second, this same extensive experience and ease of manipulation permits modification of E. coli strain K12 and the vectors for the purpose of establishing biological containment. The strain, as well as various possible vectors can, to some degree, be made to behave to order by the use of classical genetics techniques, not by recombinant DNA methodology.

It should be pointed out that the guidelines also discuss arguments against the use of E. coli K12, in particular the intimate association of

various strains of E. coli with humans. For this reason the guidelines urge that efforts be made to develop alternate bacterial host-vector systems. For this reason also, the guidelines recommend the cautious use of E. coli K12 host-vector systems.

The nature and manner of achieving containment with this system is described in the guidelines, and by reviewing this description in some detail, the general principles of biological containment will also become clearer.

The next slide (3) summarizes some relevant properties of the host

Slide 3

E. coli Strain K - 12

Appears to be harmless itself.

Does not usually colonize normal bowel.

Does not multiply significantly in alimentary tract.

Does appear in feces after feeding.

Has opportunity to transfer vectors to normal flora
during passage through alimentary tract.

cells E. coli K12. The bacteria appears to be harmless itself; it does not usually establish itself in the normal bowel, and it does not multiply significantly in the alimentary tract. These facts suggest that accidental ingestion of a small number of bacteria by a lab worker would not result in extensive spread of a bacterium outside the laboratory.

The normal situation may be altered when people are either taking antibiotics or have certain abnormal digestive conditions, and it is recommended that such individuals refrain for the duration of the abnormal situation from doing experiments with these systems.

However, while E. coli K12 does not establish itself as a growing strain in the normal bowel, it does remain alive during its passage through the tract. During the passage time, there is the possibility that the

vector containing the foreign DNA may leave the original K12 host cell and enter another E. coli cell, perhaps one of the related strains normally found in humans.

As you have already heard this morning, the extra chromosomal elements that will be used as vectors can be transferred from one cell to another during mating. Therefore, in estimating the containment of the recombined DNA itself, we need to discuss this transfer process and the likelihood that it will occur.

May I have the next slide (4), please?

Slide 4

E. coli Plasmids as Vectors

Nonconjugative plasmids:



Conjugative plasmids:



But:



As Dr. Berg pointed out, two kinds of extra chromosomal elements can be used as vectors in E. coli K12 systems. This particular slide deals with plasmids. They are shown here as small circles, and the chromosomal DNA is represented in each case by the egg-shaped structure.

Certain plasmids are called nonconjugative because, as shown here, they are unable to be transferred from one cell to another, as indicated by the X here. Because of this they would not be able to leave the original K12 cell were it to enter the alimentary tract. For this reason the guidelines recommend the use of nonconjugative plasmids as vectors in recombinant research.

Another type of plasmid shown here by a squiggly circle is call conjugative since it can be transferred to another cell, and the use of conjugative plasmids is therefore discouraged. However, the situation is somewhat more complicated as shown in the group of reactions at the bottom of the slide.

Cell A contains a conjugative, that is a squiggly plasmid, and can transfer it to Cell B, which in this case already contains a nonconjugative plasmid, so that Cell B winds up with both types of plasmids. The presence of the conjugative plasmid gives Cell B now the ability to transfer not only the conjugative plasmid but the nonconjugative one as well to a third Cell, C. Cell C might be one of the E. coli types normally residing in the bowel, and therefore the use of a nonconjugative plasmid does not totally assure containment of the recombinant.

In order to evaluate a particular plasmid-E. coli combination as to containment, we need to ask the likelihood that this series of reactions will occur. While we are missing some relevant information, what data are available suggest that the probability can be quite low, depending on the particular nonconjugative plasmid that is used.

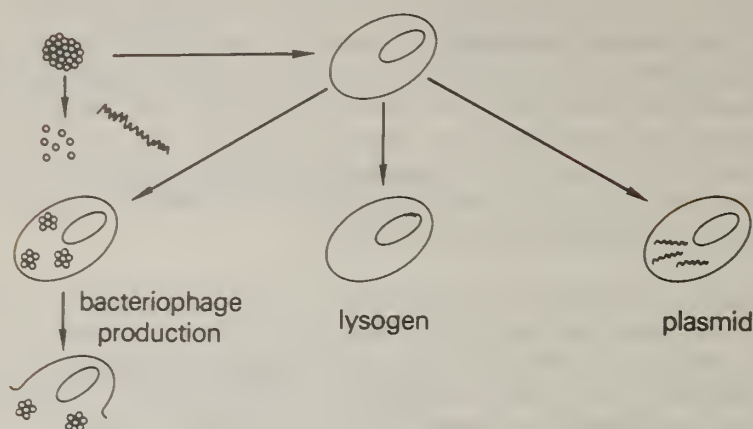
With certain known and useful plasmids, the entire series of events shown on the bottom of the slide occurs at frequencies that are less than one in 10^9 --that is, one in one thousand million. This is the first time I have used this terminology, but it occurs throughout the guidelines. For those of you who are unfamiliar with it, I have summarized it on the board. The number 10^9 is a one followed by nine zeroes. The number 10^8 is a one followed by eight zeroes. It is just much easier to use this terminology, so that as I use it, and if I forget to tell you exactly what it is in more straightforward terms, you can look at this.

Okay, so the events involving the transfers that I showed you at the bottom of the slide appear to occur at frequencies that are less than one in 10^9 or one in one thousand million, and in fact they are usually undetectable under laboratory conditions designed to resemble natural conditions.

Host-vector systems made up of E. coli K12 and such plasmids therefore appear to have only very limited ability to spread recombinant DNA molecules.

Now, viruses that infect E. coli, which are called bacteriophage, are another source of DNA that is useful for recombinant DNA experiments. Some relevant properties of bacteriophage are summarized on the next slide (5).

Bacteriophage particles which are shown here are made up of small protein molecules surrounding a DNA molecule, which is shown here as the squiggly line. The genes characteristic of the bacteriophage are in this DNA, and it is to this vector that a foreign DNA might be joined. The bacteriophage particle or the DNA can enter a sensitive E. coli K12 cell and one of three things can happen.

Slide 5*E. coli* Bacteriophage as Vectors

The DNA and the protein can be replicated in the cell forming new viral particles, and the cell can open and release those particles. Alternatively, the DNA of the bacteriophage can become an integral part of the chromosomal DNA of the bacteria, a process called lysogen formation. Or the bacteriophage DNA can establish itself as a plasmid within the bacterial cell. Therefore, in discussing biological containment for systems using bacteriophage DNA as a vector, these three possible modes of escape and perpetuation of a recombinant bacteriophage DNA must be considered.

The next slide (6) summarizes current estimates for containment of such systems as described in the guidelines. The estimates are made for a particular bacteriophage of *E. coli*, which is called lambda, since this widely studied bacteriophage is most likely to be used for recombinant experiments at the present time.

Considering first information relevant to escape of the bacteriophage particle, lambda is sensitive to the acidity of the stomach and is likely to be destroyed there. Normal intestinal strains of *E. coli* are usually not susceptible to infection by lambda, and in fact, susceptible strains are rare in nature. Further, in at least one case, ingestion of 10^{11} lambda particles yielded no detectable lambda in resulting feces.

Lambda bacteriophage are also readily destroyed by drying, as might happen if the particles got into the air.

Slide 6Containment of *E. coli* Bacteriophage (λ)

As a bacteriophage

1. λ is sensitive to acidity of stomach.
2. Normal gut strains of *E. coli* usually not susceptible to λ infection.
3. Susceptible strains of *E. coli* are rare in nature.
4. Ingestion of 10^{11} λ particles yielded no detectable λ in human feces.

As a lysogen

1. Frequent event (1 to 10^{-1})
2. λ mutants lacking ability to lysogenize.
(frequency 10^{-5} to 10^{-6})

As a plasmid

1. Frequency about 10^{-6} .

The second mode for dissemination of genes recombined into lambda DNA is integration into the chromosome of the DNA as a lysogen. This is a frequent end result of infection of *E. coli* by lambda. There are, however, known mutant varieties of lambda which lack the genes necessary for lysogen formation, and with such bacteriophage, the frequency of integration into the host chromosome is reduced to 1 in 10^5 or 1 in 10^6 -- 10^6 being 1,000,000.

The third mode, the conversion of lambda DNA to a stable plasmid, is also a relatively unlikely event occurring at a frequency of about one in a million. Considering then the properties of *E. coli* K12 as well as those of the existing useful plasmid and bacteriophage vectors, the proposed guidelines conclude that using such host-vector systems, recombinant DNAs are unlikely to be spread to humans by the ingestion or dissemination of a few hundred or thousand bacteria such as might be involved in laboratory accidents, given the standard microbiological practices. Therefore, these existing systems, and combinations of *E. coli* K12 with other similar vectors, plasmid vectors, and bacteriophage, are judged to offer a moderate level of biological containment and are defined as the lowest level of biological containment for experiments with *E. coli* systems. This level is called EK1.

As for the physical containment levels, increasing numbers specify increasing levels of containment for the *E. coli* systems. The next level, called EK2 is summarized on the next slide (7).

Slide 7

EK2: high level of biological containment.

Modification of existing *E. coli*
K12 and plasmids and
bacteriophage

Modification by classical
genetic techniques.

Containment capability demonstrated
by suitable laboratory tests.

**MODIFICATIONS TO YIELD A 'HOST-VECTOR' SYSTEM SUCH
THAT FREQUENCY OF SURVIVAL OF THE 'FOREIGN' DNA
FRAGMENT WILL BE 10^{-8} OR LESS, EXCEPT UNDER SPECIAL
LABORATORY CONDITIONS**

(Responsibility for certification of putative EK2 systems lies with the
NIH Recombinant DNA Program Advisory Committee)

EK2 host-vector combinations afford a high level of biological containment and are obtained by the modification of both *E. coli* K12 cells and relevant plasmids and bacteriophage. The modifications will be achieved by classical genetic techniques, and the level of containment must be demonstrated by suitable laboratory tests.

More specifically, the guidelines state that the system of modified *E. coli* K12 hosts combined with modified vector must be such that the likelihood of escape and survival of the foreign DNA fragment in environments approximating natural environments will be one in 10^8 --that is, one in a hundred million or less.

The recombinant is thus designed to survive and grow only under very fastidious laboratory conditions. While not stated in the guidelines, the NIH Recombinant DNA Molecule Program Advisory Committee has taken on the responsibility for certifying putative EK2 systems. At this time none have been certified, although it is anticipated that several systems will be certified in the very near future.

Various examples of the types of necessary modifications are suggested in the guidelines, specific modifications for both *E. coli* itself and for the vectors.

One additional level of contained *E. coli* host-vector systems is defined and is called EK3. The EK3 systems are essentially similar to these EK2 systems, but in order to qualify as EK3, the containment properties of EK2 systems must be demonstrated not only by microbiological and genetic analysis, but by tests in animals and other relevant environments. EK3 systems are not presently available.

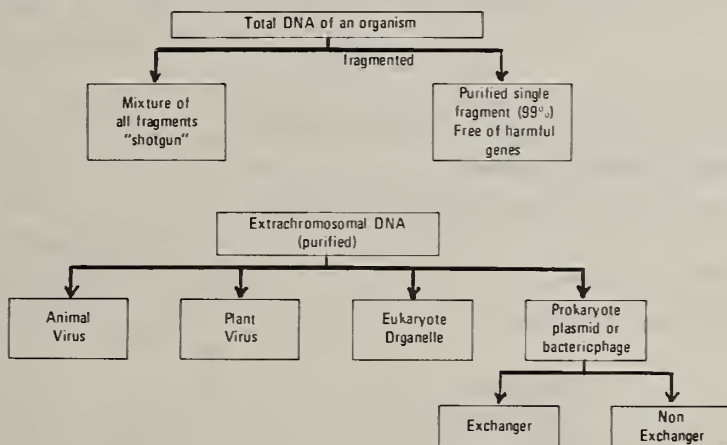
May I have the lights, please?

Having defined the several levels of physical containment, and biological containment, we can now consider the specific recommendations for experiments using the *E. coli* K12 host-vector systems. Each type of experiment is assigned both a physical containment level, that is a P-level, and a biological containment level, that is an EK-level, and the particular combination of the two reflects the severity of the estimated potential hazard.

The guidelines are organized for the *E. coli* systems according to the source and type of foreign DNA, as outlined in the next slide (8).

Slide 8

Experiments with *E. coli* "Host-Vector" Systems
Nature of the 'Foreign' DNA



The sample of DNA containing essentially all the genetic information of an organism can be isolated and fragmented by a restriction endonuclease. If the entire mixture, containing all the fragments, is used, the experiment is referred to as a "shotgun," and will call for certain levels of containment.

Experiments involving such mixtures of DNA fragments are assumed to be of relatively high potential hazard because of the greater likelihood of dangerous and unknown genes being introduced into the recipient cell.

On the other hand, a particular fragment may be purified from the mixture and used for joining to the vector DNA. Purified fragments containing mainly genes whose properties are known and are not harmful offer less potential hazard than a shotgun experiment. In some instances the foreign DNA will itself be derived from extra-chromosomal genetic elements. Such extra-chromosomal elements include the DNA of animal viruses, of plant viruses, of eukaryote organelles such as mitochondriaer chloroplasts and, finally, prokaryotic plasmids or bacteriophage of the same type used as the vectors. Each of these cases is treated separately in the guidelines.

The word prokaryote refers to some simple one-celled organisms which do not contain a well-defined nucleus. The word eukaryote refers to all other living species, and these all contain their genetic information within a clearly-defined nucleus. Furthermore, the prokaryote sources here are treated separately depending on whether the source of foreign DNA is an organism that does or does not exchange genetic information with E. coli in nature.

The next few slides will summarize quickly the recommended containment for the various categories summarized here.

May I have the next slide, please (9)?

Slide 9

Guidelines for Experiments with *E. coli* "Host-Vector" Systems. I

'Foreign' DNA = Mixed Fragments = "Shotgun"

Foreign DNA Source	Containment	
	Physical	Biological
Eukaryotes:	Primates	P3 or EK3
		P4 or EK2
	Embryonic	P3 EK2
	Other Mammals	P3 EK2
	Birds	P3 EK2
	Cold Blooded Vertebrates	P2 EK2
	embryonic	P2 EK1
	Lower Eukaryotes	P2 EK1
	if pathogenic	P3 EK2
	Higher Plants	P2 EK2
	if pathogenic	P3 EK2

First we consider shotgun experiments where the foreign DNA is a mixture of fragments derived from eukaryotes. The recommended physical and biological containment is listed for various possible DNA sources, and both must, of course, be used. They complement each other. For example, DNA from primates requires the most stringent containment, since the estimated potential hazard, either from genes that might function in humans with untoward effects or from pathogenic viral DNAs residing in primate tissue, is judged to be the most serious.

The experiments require either P3 and EK3 or P4 and EK2, and it should be recalled that only the latter combination, P4 and EK2, is feasible at present, and even then, only at a limited number of P4 facilities. That is assuming the near-certification of EK2 host-vector systems.

Another point of interest is that in two instances, primates and cold-blooded vertebrates, containment requirements are lower if the DNA is isolated from embryonic tissue or germ line material, since such material is less likely to be contaminated by pathogenic viruses or other adventitious agents than is adult tissue.

Thus, if the foreign DNA is from cold-blooded vertebrates, P2 and EK2 are required; P2 and EK1 can be used if the DNA is from embryonic or germ line tissue. In some instances--lower eukaryotes, for example--the guidelines require more or less stringent conditions, depending upon whether or not the source of foreign DNA is known to be pathogenic or might be infected with a pathogen or is known to make a harmful product. The lower eukaryotes include invertebrates, certain plants, yeast, and fungi.

The next slide (10) summarized the proposed guidelines for shotgun experiments when the source of the DNA is a prokaryotic organism. Two groups considered: first those prokaryotes which are known to exchange genetic information in nature with E. coli, and the containment recommendations are low for this group and vary with the pathogenicity of the source of foreign DNA. When the source of foreign DNA is a prokaryote that does not normally exchange genetic material with E. coli, the containment recommendations are higher.

Finally, the containment requirements are raised in situations which are termed on the slide as involving a drug problem--that is, situations where experiments may extend the range of resistance of the host cell to therapeutically useful antibiotics or disinfectants.

We now consider the situation where the foreign DNA is still derived from chromosomal DNA, but it is purified prior to joining to the vector.

May I have the next slide, please (11)?

Slide 10Guidelines for Experiments with *E. coli* "Host-Vector"
Systems II.

'Foreign' DNA = Mixed Fragments = "Shotgun"

Foreign DNA Source	Physical	Containment	Biological
Prokaryotes:			
"Exchangers"			
Class 1 (CDC)	P1		EK1
Class 2 (CDC)	P2		EK1 – EK2
Plant pathogens	P2		EK1 – EK2
"Non-exchangers"			
Class 1 (CDC)	P2		EK1
Class 2 (CDC)	P3		EK2
Plant pathogen	P3		EK2
If 'drug' problem	P2*		EK2*

*Or higher, depending on virulence.

Slide 11Guidelines for Experiments with *E. coli* "Host-Vector"
Systems IV.

"Foreign DNA" = Purified fragment of chromosomal DNA

- a) greater than 99% pure
- b) absence of harmful genes

Then:

Decrease Physical Containment one step

(P3 → P2)

or

Decrease Biological Containment one step

(EK2 → EK1)

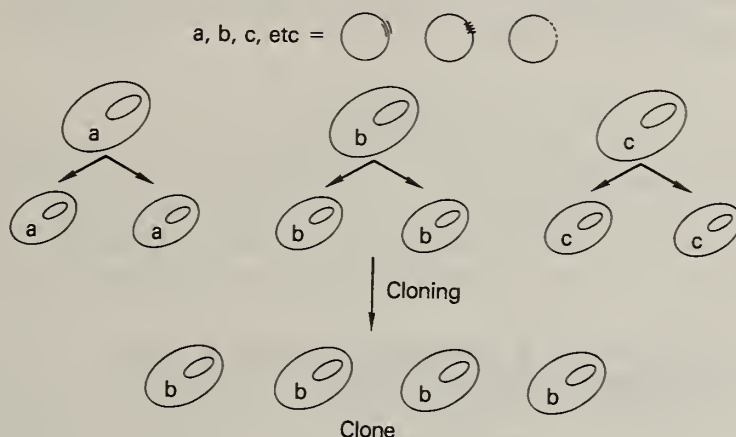
from containment required for "shotgun"

The guidelines define purified as meaning that more than 99 percent of the purified DNA by weight represents the desired fragment, and further they require evidence that no harmful genes are present. Under such circumstances the investigator may lower the containment conditions from those recommended for shotgun experiments with DNA of the same source, either by one step in physical containment or by one step in biological containment.

In these experiments where the foreign DNA is purified before making the recombinant, all of the offspring of the recipient cell will ideally contain only a single kind of recombinant molecule, one containing the original purified fragment of DNA. It is distinguished from the case of shotgun experiments where the foreign DNA was a mixture of many fragments, as shown on the next slide (12).

Slide 12

"Shotgun" Experiment



During the recombination in the shotgun experiment, each of the fragments, listed here as A, B, C, et cetera, can be combined separately with molecules of a vector and a large number of different recombinants indicated here are generated. Each recombinant DNA molecule may then enter a different individual cell in the population of host cells used as recipients. When each of these divides, each of its offspring will, like itself, contain the recombinant that was originally received by that parent, so all of the offspring of this cell will contain recombinant A, all of the offspring of this, recombinant B, and so forth.

There are a variety of techniques available for isolating from such a mixture only those cells which contain a single desired recombinant, and these procedures are referred to as cloning, and the isolated population a

clone. The definition of a clone is, in fact, a population, all of which are derived from a single parent.

In the experiment on this slide the clone contains only recombinant B. Cloned populations are judged to be inherently less potentially hazardous for reasons similar to those outlined for the case when purified DNA fragment is used for the joining. Consequently, the containment required once the cloning procedure has been carried out is less than the containment required for the original shotgun experiment.

The cloning of a desired fragment will, of course, as pointed out by Dr. Berg before, be one of the normal aims of many recombinant experiments.

As shown in the next slide, (13) the guidelines state that the cloned recombinant DNA must be characterized in indicated manners, be free from harmful genes, and also that approval must be obtained from the agency supporting the work before the containment can be lowered to the categories which are shown here. There are two categories, one for DNA which originally came from species which exchange information in nature with E. coli and another category for those that don't.

Slide 13

Guidelines for Experiments with *E. coli* "Host-Vector" Systems III

Cloned Recombinant DNA

- a) rigorously characterized
- b) absence of harmful genes
- c) approval from granting agency

Then:

"Exchangers"	P1	EK1
"Non-exchangers"	P2	EK1

May I have the next slide, please (14)?

Slide 14

Guidelines for Experiments with *E. coli* "Host-Vector" Systems V.

"Foreign" DNA = Extrachromosomal DNA elements (purified)

"Foreign" DNA Source	Containment		
	Physical		Biological
Animal Viruses	P4	or	EK2
	P3		EK3
if cloned, free of harmful genes	P3		EK2
Plant Viruses	P3	or	EK1
	P2		EK2
Eukaryote Organelle:			
primates	P3	or	EK1
	P2		EK2
Other	P2		EK1
Prokaryote (plasmids and phage)			
"Exchangers"			
non-pathogens	P1		EK1
pathogen		As for shotgun (P2 EK1)	
"Non-exchangers"		As for shotgun (P2 EK1)	
If 'drug' problem	P3		EK2

The final group of experiments utilizing E. coli host-vector systems are those in which the foreign DNA is itself from an extra-chromosomal element; and as I indicated before, it is assumed that such DNA is purified away from the chromosomal DNA prior to the recombination. Various possible sources of extra-chromosomal DNA are listed, and the recommended combined containment given.

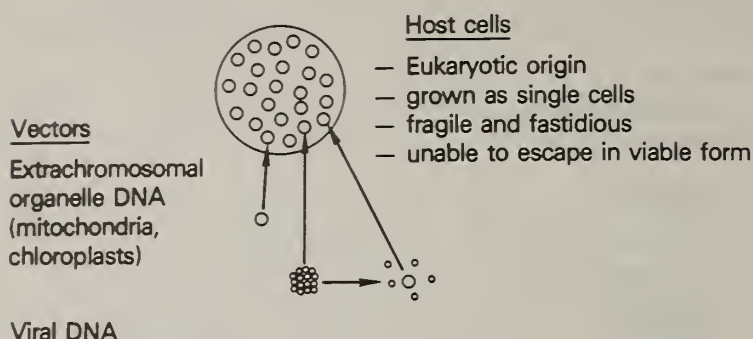
For example, DNA from animal viruses requires P4 physical containment and an EK2 host-vector system, or alternatively, P3 physical containment and an EK3 host-vector system. If the recombinants have been purified by cloning and shown to contain only harmless regions of the viral genome, then experiments can be moved to P3 and EK2.

Many of the recombinant DNA experiments currently being talked about and planned involve, rather than the use of E. coli host-vector systems, systems in which the host cells are eukaryote cells grown as single cells under special laboratory conditions. This is shown on the next slide (15).

In the middle of the slide is a plate on which such cells are growing. The vectors that are available for such experiments include extra chromosomal DNA elements normally found in eukaryote cells which are here called organelle DNA, and also the DNA of viruses that infect particular cells of interest. Given the current state of technology, viral DNAs are most likely to be used as vectors in the near future.

Slide 15

Experiments with Eukaryotic Host-Vector Systems



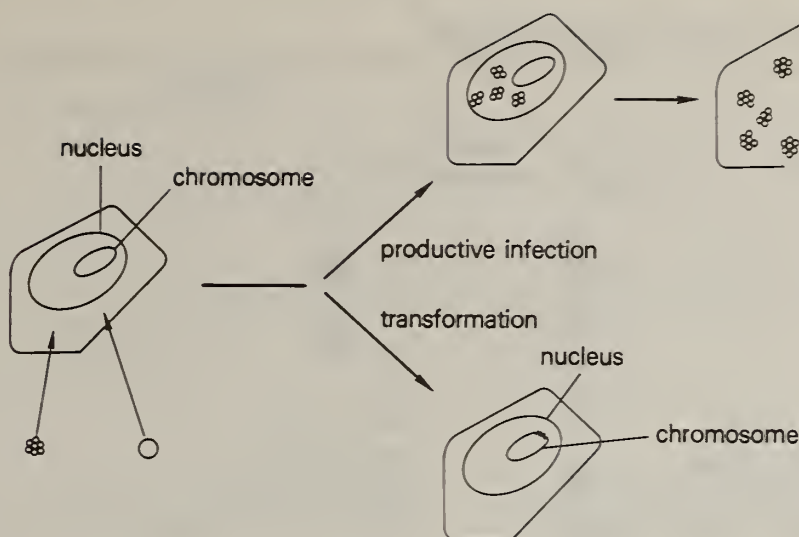
We will talk first about animal cells. These host cells might typically be derived from hamsters, mice, monkeys, or humans. The cells themselves are fragile and fastidious, as is well-known by anyone who has spent time trying to grow them. There is little or no chance that a living cell could escape from a laboratory in the way that E. coli might. Therefore, the containment considerations focus on the virus.

Animal viruses can, of course, be carried out of laboratories and can be spread if laboratory workers become infected. As shown in the diagram at the bottom, these viruses, like the bacteriophage, are composed of a DNA molecule, shown here as a circle, the largest circle, encased in a protein coat shown by the small circles. The virus itself, as well as the isolated DNA can enter and infect cells.

May I have the next slide (16), please?

As with the bacteriophage, several alternative situations may result upon infection of animal cells with animal viruses or viral DNA. The virus or viral DNA may enter the cell, use the cell as a factory for producing more viral DNA and protein, and finally, new viral particles may be released from a destroyed cell.

Alternatively, the viral DNA may become joined to the original chromosomal DNA of the host cell, and thereby be passed to every offspring of the cell in a process which is called transformation.

Slide 16

As shown in the next slide (17), there are two animal viruses whose DNAs are now technically useful vectors. Cleavage of the DNA of these viruses by restriction endonucleases has been studied extensively, and it is feasible to insert a piece of foreign DNA into such vectors. Such a recombinant DNA may now infect appropriate cells, new viral particles are produced, and they therefore would contain DNA containing a replica of the original foreign fragment that was introduced.

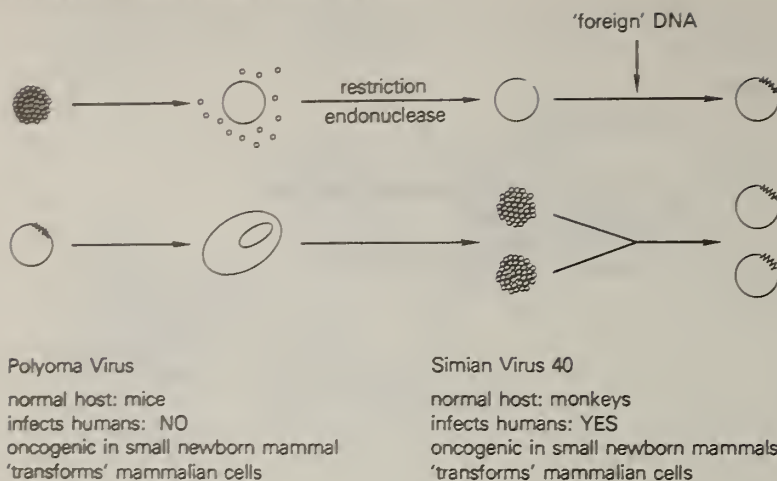
The two viruses are called polyoma and Simian Virus 40, and a few of their relevant properties are listed below. In their respective normal hosts, mouse for polyoma and rhesus monkeys for Simian Virus 40, neither virus causes a known disease. Polyoma does not infect human cells grown as single cells in the laboratory, and also does not appear to infect humans, since humans exposed to polyoma do not produce antibodies.

Simian Virus 40 does infect both human cells grown as single cells in the laboratory and whole human beings, as evidenced by the act of production of antibodies. This was the virus which was found to contaminate the early Salk polio vaccines, and millions of people were inadvertently inoculated with it in the middle '50's. To date there is no indication that the recipients of the vaccine have suffered any related difficulties.

Both of these viruses are oncogenic--that is, they cause tumor formation in newborn --small laboratory mammals--and both can transform a variety of cells of mammalian origin. They are classified as low-risk oncogenic viruses by the National Cancer Institute, and the viruses themselves, not recombinants, must be handled under conditions equivalent to P2.

Slide 17

Presently Useful Eukaryotic Vectors



Because Simian Virus 40 infects human beings, and also because Simian Virus 40 and related viruses have been isolated in connection with several human disease states, the proposed guidelines assume that polyoma affords the higher level of biological containment, and therefore more stringent physical containment of Simian Virus 40 is required than for polyoma.

An increase in the level of biological containment afforded by the two unaltered viral DNAs can also be obtained. This is shown on the next slide (18).

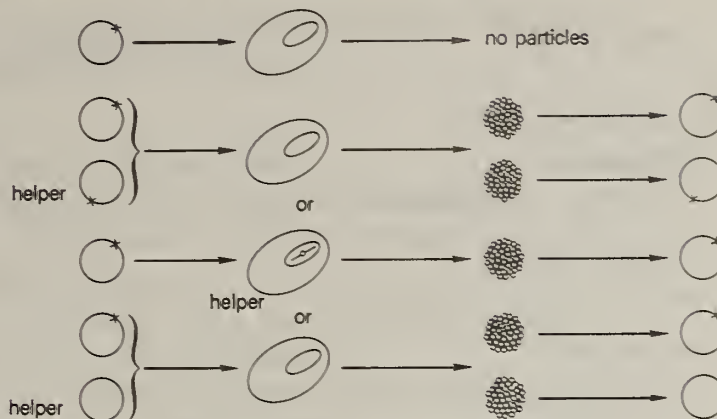
The guidelines require that the viral DNA used for recombination with a foreign DNA must itself be defective. Such defective viruses are unable to reproduce efficiently. They are available and they result from changes or mutations in the viral DNA. A defect is indicated schematically here by an X in the circular DNA of the virus. Upon infecting a cell, no new viral particles can be made. For the purpose of an experiment which requires the production of viral DNA, infection is carried out in the presence of a helper virus, which supplies the genes for the missing function.

As shown in the next set here, this could be another defective which is defective for another gene somewhere else on the DNA, and between these two viral genomes, all the necessary genes are provided, and two types of viral particles can be produced containing either of the two original defective genomes. Or, as shown in the next line, an intact form of the defective gene might previously have been inserted into the chromosomal DNA of the recipient cell, thus also supplying the missing function and permitting the production of the desired defective virus.

Slide 18

Guidelines for use of Polyoma and SV40 as Vectors

I. Vector must be a defective genome



Finally, the helper could be a complete, intact virus. Any one of these alternatives allows the production of the defective viral particles, or of the defective containing a recombined foreign DNA; but the defective particles produced would, should they escape, have limited ability to infect susceptible species in a significant manner.

In certain kinds of experiments no production of viral particles is required, and no helper may be needed. Biological containment is inherently greater in the absence of viral particles, since, as pointed out before, the cells themselves are relatively easy to contain.

Keeping in mind these aspects of biological containment, we can look at the recommendations for physical containment with these experiments, and they are summarized on the next slide (19).

The particular P-level depends on the source of the foreign DNA, on whether a defective polyoma or a defective SV40 (Simian Virus 40) is the chosen vector, and finally on whether or not virus particles are produced.

Looking first at the experiments with defective polyoma vectors, under conditions where viral particles are produced, if the foreign DNA is itself from a non-pathogenic agent, P3 conditions are required, even if the DNA fragment was purified first and does not contain harmful genes.

If the foreign DNA is from an organism with low pathogenicity, P4 must be used until such time as suitable tests indicate that only harmless genes are present.

Slide 19Guidelines for Use of Polyoma and SV40
as Vectors

2.

'Foreign' DNA	Physical Containment	
	Polyoma	SV40
With Virion Production		
non-pathogen	P3	P4
if purified, cloned, harmless	P3	P3
low pathogenicity	P4	—
No Virion Production		
non-pathogen	P3	P3
low pathogenicity	P3	—

Still considering polyoma vectors, P3 conditions are required for experiments in which no virus particles are produced. When SV40 is the vector, and virus particles are produced, P4 conditions must be used if the foreign DNA is from a non-pathogenic organism, and experiments can be moved to P3 only after extensive and specified kinds of purification and demonstration that no harmful genes are present.

SV40 cannot be used at all for experiments with DNA from pathogenic organisms. When no SV40 virus particles are produced, experiments with recombinants derived from non-pathogenic agents can be carried out in P3 conditions.

The guidelines also contain recommendations for experiments in which plant cells would serve as hosts for recombinant DNA. These cells might be single plant cells grown under laboratory conditions, or seedlings or plant parts, or whole small plants. This is, in fact, the only instance where the guidelines address the question of recombinant DNA experiments with whole multicellular organisms.

Vectors for use in experiments with plants include plant organelle DNA such as the DNA of chloroplasts and DNA of viruses of low pathogenicity. These vectors offer moderate levels of biological containment, and the guidelines suggest the physical containment outlined on the next and last slide (20). As before, this is organized according to the source of the foreign DNA. If the foreign DNA is derived from a species in which the vector DNA is known to be able to reproduce, P2 conditions are required,

unless the source of foreign DNA is pathogenic, or produces products dangerous to plants; then P3 is required.

Slide 20

Experiments with Plant 'Host-Vector' Systems 2.

Source of 'foreign' DNA	Physical Containment
Species in which vector can replicate	P2
if harmful products possible	P3
Other species	
Foreign DNA purified (99%), and no harmful genes	P2
Foreign DNA not purified, and no harmful genes	P3
Foreign DNA contains harmful genes	P4

If the foreign DNA is derived from a species in which the vector is not known to replicate, that is, all other species, and thus the vector is in a sense also a foreign DNA, then more stringent requirements govern, and they vary from P2 to P4 depending on whether the DNA is purified and whether it contains harmful genes. Special, somewhat more stringent requirements are recommended for experiments with whole plants, and also the guidelines describe specific modifications of the physical containment specifications in order to make them applicable to work with plants.

May I have the lights, please?

Theoretically there are a variety of organisms, both prokaryotes and lower eukaryotes--that is, fungi and yeast--which will be interesting and useful hosts for experiments with recombinant DNAs. Some may offer the special advantage of not infecting humans or important animals, or important ecological niches. However, a variety of technical developments are needed before useful vectors are available for these systems. The growth characteristics of such hosts indicate that the containment problems will be like those for E. coli K12 host-vector systems. The guidelines urge the development of these systems, and point out that the detailed recommendations made for E. coli K12 systems can be used as a guide in determining biological and physical containment requirements for these systems when that is required.

The advisory committee has included in the proposed guidelines specific recommendations concerning implementation in the instance of work supported by research grants.

First, the principal investigator is given extensive responsibilities. The principal investigator is most likely to be either the person who is doing the experiments or in groups working together, a senior scientist who might have under his direction graduate students, post-doctoral fellows, technicians and so forth. This individual has the responsibility to assess any potential biohazards, to institute appropriate safeguards and procedures, to minimize the effect of possible accidents by planning, to train and inform all personnel, and all of these on a continuing basis.

Thus, the primary responsibility for conducting experiments according to the guidelines is his hands.

Further, in applying for grants to carry out experiments on recombinant DNA, the investigator must include an estimate of the potential biohazards as well as a statement as to the containment procedures that will be used. Should these procedures include P3 or P4 conditions, the application must include certification as to the existence and availability of facilities which meet the definitions of P3 or P4 as described in the guidelines.

Therefore, the guidelines also indicate that institutions in which recombinant DNA experiments are carried out must establish biohazard committees which can serve to examine equipment and facilities and to certify their compliance with the requirements for P3 and P4 containment.

Such committees will also serve as a source for advice and reference on physical containment facilities, on the properties of biological containment systems, and on the training of personnel.

The guidelines specifically state that it is not the responsibility of such committees to determine either the scientific quality of proposed experiments or the containment conditions required.

According to the proposed guidelines, review of the investigator's judgment concerning the extent of potential hazard and the required containment would be by peer groups during the normal scientific review of the application. The guidelines leave flexible the question of resolving any differences between the evaluation of the investigator and that of the peer group, and presumably this might be done informally through the executive secretary of the study section. The guidelines do state, however, that in instances where resolution of differences cannot be made the matter should be referred to an appropriate committee.

The proposed guidelines further specify that in those instances where plans are made to initiate experiments on recombinant DNA under already

existing grants, the supporting agency can review the estimates of hazards, the proposed containment facilities and certification, and decide whether or not to endorse the proposal without peer review, unless there are unresolved questions concerning the proposal.

That concludes the summary of the guidelines.

DR. FREDRICKSON: Thank you very much, Maxine. That was a superb job. I think we will ask you to provide us with some glossies of those slides. There may be many who would be interested in having them.

Your presentation by no means being the cause--I am sure that some members of the committee are getting a little restive at the didactic tone of the meeting. I would describe some modification that has already been made, and that is that we have drastically cut Dr. Barkley's presentation this afternoon, eliminated the movie, and substituted a few slides for it in order to be sure that there is ample time for discussion.

We are not running badly, however, from our schedule, and I do want to go ahead then with the last two presentations scheduled for this morning.

We have asked and put an unpleasant restraint on both Drs. Hogness and Curtiss in the amount of time that we were able to allot to them, and I hope they will be able to conform to that restraint. We are very eager to hear from each of them because they represent at least in the beginning, in our understanding, some differences in the point of view within the committee as the guidelines were developed.

First we would like to ask Dr. David Hogness, who is a Professor of Biochemistry at Stanford, if he would give us, then, a summary of his early impressions about these guidelines and such differences as may have existed during that drafting.

Dr. Hogness, may we count on you for just 10 minutes?

DR. HOGNESS: Sure, just ring a bell.

DR. FREDRICKSON: I probably shall.

DR. HOGNESS: In commenting on the formulation of these guidelines, it might be useful by way of background to indicate that we have been constructing and cloning recombinant DNA molecules in my laboratory since 1973, and that on the other hand, I have been associated with most of the successive steps that resulted in the guidelines before you.

In reviewing these steps I have been struck by certain trends that I view with some concern, and which I should like to emphasize here.

One of these trends has consisted of the general tendency to increase the levels of containment applied to experiments with recombinant DNAs, and this can be illustrated by considering the fate of certain shotgun experiments as they have passed through the successive guidelines, starting with the letter that appeared in Science about one and a half years ago.

The experiments I should like to consider are those in which random segments of DNA obtained from eukaryotic chromosomes are cloned in E. coli. In the Science letter, these experiments were not included within the group for which we asked voluntary deferment. Rather, we limited our comment to the general admonition that they should be carried out with caution as they might engender certain hazards, some of which we noted. the general intent was to stimulate awareness in the scientific community of the potential dangers.

In the next step, these experiments were divided into certain classes, and levels of containment were recommended for each class, first by a working committee whose job it was to prepare a report for consideration at the Asilomar conference, and then by the organizing committee of that conference. Certain increases in containment levels occurred during that process. For example, the working committee recommended that experiments with the mammalian DNAs, including those from primates, be carried out under conditions equivalent to P3 plus EK1. You can remember these terminologies. These were increased to a containment comparable or slightly higher than P3 plus EK2 by the organizing committee--a classification that was ratified by the conference participants.

In subsequent consideration by the NIH Advisory Committee, the containment conditions for these mammalian experiments were left at P3 plus EK2 in the Woods Hole guidelines, but were again increased last December at La Jolla. Thus, experiments with primate DNAs were separated from the general mammalian class and assigned P4 plus EK2, or P3 plus EK3 containment levels, while the remaining experiments with mammals were retained at the P3 plus EK2 level.

In a similar move, experiments with cold-blooded vertebrates, which had remained constant at P2 plus EK1 from Asilomar through Woods Hole were increased one level, that is to P2 plus EK2 at La Jolla.

This trend to increase containment levels would clearly not be of concern to me were it based on data indicating that these experiments were potentially more dangerous than we had previously imagined. However, this was not the case. Indeed, the data that has accumulated since the Science letter was published argue in the opposite direction. These data include the finding that E. coli K12, the strain of E. coli used in these experiments, is highly attenuated. It does not colonize in normal bowels of humans or

other tested mammals, and more recently it has been shown that it does not even become pathogenic when containing certain plasmids that normally confer pathogenicity to more robust strains of E. coli.

In addition, it has recently been shown that recombinant DNAs consisting of lambda phage containing any one of thousands of different eukaryotic DNA segments, in this case from yeast, never grow as well as the parental lambda vector. That is, they are selected against in a growth race against the normal DNA.

Finally, experiments in our laboratory and in John Carbon's laboratory indicate that the probability that the eukaryotic genes are expressed in E. coli is quite small--much less, for example, than is the probability of expression by prokaryotic genes in E. coli.

Why, in the face then of this evidence, were the containment levels continuously increased? I suggest that one of the important factors has been a preoccupation and speculation as to the conceivable hazards of these experiments without a corresponding concern about their benefits. In considering this balance between benefits and hazards, I should like to distinguish between areas of fact and of speculation. Some of the benefits result from a fact that can be simply stated. By the use of these shotgun experiments, one can isolate individual genes and examine in molecular detail their structure, function, and arrangement in the complex chromosomes of higher organisms. Without that use, one cannot.

This is an important fact, because such an examination is critical to a determination of how these genes are reproduced and of how they are turned on and off in different cells. This determination is, in turn, of critical importance to our understanding of cell development, both normal and pathological.

By contrast, we enter the speculative arena when we consider either the directly-applied benefits or the hazards of such experimentation. I do not wish to belittle either area of speculation. The applied benefits that we can imagine are indeed considerable, ranging from the economical production of medically useful proteins, such as specific antibodies, to significant increases in our food supply.

The hazards that one can imagine are certainly not trivial, or we should not be here today.

However, one's evaluation of each involves guesswork, and whatever the balance obtained between them, the result must be stacked up against the hard fact that I referred to before, namely that these experiments allow a dramatic increase in our knowledge about one of the most important characteristics of life--that is, its genetic structure and function.

In the present climate of opinion I think that this factor of a gain in knowledge has been underevaluated, and that our fear of the unknown has been overly emphasized, with the result that there has been, in my opinion, an overshoot in establishment of too restrictive conditions for containment in certain areas of these guidelines to which I have referred.

For example, it was my opinion when I was a member of the Asilomar working group that sufficiently strict conditions of containment for the mammalian shotgun experiments are P3 plus EK1, or with the advent of EK2 vectors, P2 plus EK2. I remain convinced that this is the case.

In this regard, you should note that P3 conditions are not trivial. They are, for example, those recommended by NIH for the handling of the most virulent and pathogenic bacteria known to man, bacteria such as Pasteurella pestis. Even P2 conditions are considered adequate for bacteria with very significant pathogenicity, such as those responsible for diphtheria and anthrax.

My comments on this subject would be incomplete if I did not note that though I disagree with much in the guidelines, I am glad that our committee has finally come forth with a document, simply because we now have, and you all have, something definite to refer to, something we can criticize, and something, I hope, we can change.

I should like to conclude with a final fact that I shall leave to your own interpretation. In the past two and a half years, we have, in my laboratory, cloned and handled tens of thousands of different recombinant molecules containing DNA from fruit flies under the prescribed conditions, P2 plus EK1. In that time, our monitoring of the personnel involved in these experiments indicates that none have suffered any ill effects above the typical colds and one sprained back.

By quoting the statistics I do not mean to indicate that I believe no dangers exist. Rather, I give it in closing to emphasize that we have as yet no evidence that the hazards that we can imagine exist.

DR. FREDRICKSON: Thank you very much, Dr. Hogness.

We have also asked Dr. Roy Curtiss, who is a Professor in the Department of Microbiology at the School of Medicine, University of Alabama, to do the same as Dr. Hogness has just done.

Dr. Curtiss? I might add that Dr. Curtiss is one of those who has been recently responsible for some of the laboratory breakthroughs in developing safer host-vector pairs.

DR. CURTISS: Thank you.

I became a vocal participant in the discussion on recombinant DNA molecule research soon after the publication of the Berg et al. letter of July 1974. I have taken a rather consistent conservative attitude since that time in believing that the potential biohazards were far more likely to be real than unreal.

I have therefore argued that one should err on the conservative side in stipulating containment conditions for any given experiment when in doubt about the potential biohazards, that terminology should be sufficiently well-defined to avoid ambiguity in interpretation, and that there should be a mechanism to assure that investigators were using the appropriate physical and biological containment systems and procedures.

I was thus reasonably satisfied with both the Asilomar guideline statement and the draft guidelines prepared by Dr. Hogness and his subcommittee for presentation to the NIH Advisory Committee at our Woods Hole meeting.

The outcome of that meeting, however, was to amend the draft to liberalize the classification of some experiments, to muddy the definition of some important terminology, and of most concern to me, to reduce the safety requirements for E. coli K12 biological containment systems, and to permit almost anyone to designate a host or a vector as meeting EK2 standards on the basis of theoretical considerations and without a requirement for data to be presented to substantiate the claim. I thus voted no on the adoption of those guidelines.

The La Jolla document which you all have, and which Dr. Singer described this morning, remedied all of these problems, and I was thus pleased and very much relieved at the end of that meeting to be able to cast a yes vote in favor of the adoption of these guidelines.

In the summer of 1974 my lab group was initiating recombinant DNA molecule research and had begun to genetically manipulate E. coli K12 strains to facilitate these endeavors. I thus conceived of the idea to also genetically alter E. coli to make it safer for this research, and communicated these ideas to my colleagues who were drafting the suggested guidelines for recombinant DNA molecule research with prokaryotic organisms to be presented at the Asilomar conference. These proposals for the design of safer hosts and vectors are contained in an appendix to our report made at that meeting.

Since Asilomar my group at the University of Alabama has worked full time in designing safer E. coli K12 hosts, and I must admit the task has been far more difficult than I or others ever imagined. Indeed, it was our difficulties last summer that caused me greatest concern about the guidelines drafted at Woods Hole.

I will now briefly describe our objectives and accomplishments during the past year in disarming E. coli K12.

First slide, please.

Slide 1

GOALS IN CONSTRUCTING SAFER E. COLI K-12

Strains should possess mutations that:

1. Increase usefulness of strains for research
2. Preclude colonization of and survival in intestinal tract
3. Preclude biosynthesis of cell wall in non-laboratory controlled environments
4. Lead to degradation of DNA in non-laboratory controlled environments
5. Cause cloning vector replication to be dependent on host
6. Preclude or minimize transmission of recombinant DNA to other bacteria
7. Permit monitoring of strain

Our goals in constructing safer E. coli K12 stipulated that strains should possess mutations that: one, increase their usefulness for the research; two, preclude colonization of and survival in the intestinal tract; three, preclude biosynthesis of cell wall in non-laboratory-controlled environments; four, lead to degradation of DNA, that is, genetic information, in non-laboratory-controlled environments; five, cause cloning vector replication to be dependent on the host; six, preclude or minimize transmission of recombinant DNA to other bacteria; and seven, permit monitoring of the strain.

We have now constructed and tested a strain which meets these specifications except for that stipulated in goal five, since development of safer cloning vectors is being worked on by other researchers.

The next slide (2) gives a description of our potential EK2 host. Strain x1776, and its derivative, x1876, which carries the pSC101 plasmid cloning vector, possess the following traits: first, mutations conferring important properties are due to deletions in genetic information, thus they are stable; two, they require diaminopimelic acid, an unusual amino acid not prevalent in nature, which is needed for cell wall biosynthesis, and

Slide 2

DESCRIPTION OF POTENTIAL EK2 HOST

Strain x1776 and its derivative, x1876, possess the following traits:

1. Mutations conferring important properties are due to deletions of genetic information
2. Require diaminopimelic acid (needed for cell wall biosynthesis) and thymidine (needed for DNA synthesis)
3. Have generation times 2-4 times longer than wild-type E. coli strains
4. Are sensitive to bile salts
5. Are very sensitive to ultraviolet light
6. Are more sensitive to many antibiotics, drugs, and detergents than parental strain from which derived
7. Cannot be productively infected with most known E. coli transducing phages
8. Are unable to inherit most conjugative plasmid types and are defective in inheriting other conjugative plasmid types

thymidine, which is needed for DNA synthesis, and also mutations that do not permit the production of mucopolysaccharides, which permit wall-less bacteria to survive in nature; three, have generation times two to four times longer than wild-type E. coli strains, and thus will not permit competition with robust strains in nature; four, are sensitive to biosalts, thus precluding survival in the intestine; five, are very sensitive to ultra-violet light; six, are more sensitive to many antibiotics, drugs and detergents than the parental strain from which derived; seven, cannot be productively infected with most known E. coli transducing phages which could act to transmit genetic information to other strains in nature; and eight, are unable to inherit most conjugative plasmid types and are defective in inheriting others under optimal laboratory conditions for detecting these events.

Next slide (3), please.

In addition to the traits listed on the previous slide, these strains: one, cannot survive passage through the intestinal tracts of rats, when 10

Slide 3

Strains x1776 and x1876:

1. Cannot survive passage through intestinal tracts of rats when 10^{10} cells suspended in milk are introduced by using a stomach tube
2. Die, degrade their DNA, and lyse in growth media lacking diaminopimelic acid and thymidine
3. Die at variable rates following drying or when suspended in water or in physiological saline
4. Cannot transmit genetic information by transduction or conjugation at detectable frequencies under any of the above-described non-permissive conditions

rates following drying or when suspended in water or in physiological saline; and four, cannot transmit genetic information to other robust laboratory E. coli K12 strains by transduction or conjugation at detectable frequencies under any of the above-described non-permissive conditions.

I should clearly indicate that I am not claiming that these strains are EK2 hosts, since all our data on testing the strain must still be subjected to the scrutiny and evaluation during peer review by scientists selected by NIH.

Lights, please.

I have recently been writing a review on the potential benefits and biohazards associated with the use of microorganisms for recombinant DNA molecule research. In the remaining few minutes, I will abstract from this review and briefly consider some estimates for the probabilities of escape, survival, and manifestation of adverse consequences of recombinant DNA molecules contained in various bacterial host-vector systems.

Next slide (4), please.

Slide 4ESTIMATING PROBABILITIES FOR ESCAPE, SURVIVAL, AND MANIFESTATION OF
ADVERSE CONSEQUENCES OF RECOMBINANT DNA MOLECULES

Probability of escape from the laboratory depends on:

1. Personnel--training and use of acceptable procedures
2. Facilities--P1 vs P2 vs P3 vs P4
3. Existence and implementation of accident plan
4. Number of investigators using the techniques

Probability of survival and perpetuation depends on:

1. Nature of bacterial host and cloning vector
2. Ecological niches occupiable by original and chimeric hosts
3. Transmissibility of recombinant DNA to other bacteria
4. Selective advantages or disadvantages conferred by foreign DNA

Probability of manifestation of adverse consequences depends on:

1. Source of foreign DNA
2. Expression of foreign DNA

In estimating the probabilities for escape, survival, and manifestation of adverse consequences of recombinant DNA molecules, one has to consider a number of factors. The probability of escape of recombinant DNA molecules from the laboratory depends on: one, the personnel, their training and use of acceptable procedures; two, the facilities, P1 versus P2 versus P3 versus P4; three, the existence and implementation of an effective accident plan; four, the number of investigators using the techniques and the number of chimeric bacteria constructed and grown. The probability of survival and perpetuation of recombinant DNA molecules in nature depends on: one, the nature of the bacterial host and the cloning vector; two, the ecological niches occupiable by the original host and the genetically modified or chimeric host; three, the transmissibility of recombinant DNA to other bacteria; and four, the selective advantages or disadvantages conferred by foreign DNA.

The probability of manifestation of adverse consequences of recombinant DNA molecules depends on: one, the source of the foreign DNA; and second, the expression of this DNA in a prokaryotic host.

On the last slide (5) I have enumerated estimated probabilities for shotgun experiments with mammalian DNA using three different host-vector systems. These estimates are based on data whenever possible, and on

Slide 5ESTIMATED PROBABILITIES^a

Host	Vector	Survival ^b	Transmissibility ^c	No selective disadvantage ^d	Exhibit adverse consequences ^d
Robust <i>E. coli</i> K-12	R100	10^{-1} - 10^{-2}	10^{-8}	10^{-2}	10^{-6}
Robust <i>E. coli</i> K-12	pSC101	10^{-1} - 10^{-2}	10^{-19}	10^{-2}	10^{-6}
EX2 <i>E. coli</i> K-12	pSC101	$<10^{-8}$	$<10^{-23}$	10^{-2}	10^{-6}

^a Assume "shotgun" experiment with mammalian DNA.

^c Per surviving escaped bacterium per day.

^b Per escaped bacterium per day.

^d Per cloned DNA segment.

conservative guesses when no data are available. Estimates on probabilities of escape from various physical containment systems are left for Dr. Barkley to discuss this afternoon.

In the first example, with a robust *E. coli* K12 host, with a conjugative R100 plasmid vector, one to ten percent of the escaped bacteria will survive each day, and one out of one hundred million of these surviving escaped bacteria should be able to transmit the recombinant DNA to some other bacterium in nature. These values assume that the escape occurs, either by accidental ingestion or by disposal down the drain.

Most foreign DNA causes a microorganism that possesses it to be at a distinct selective disadvantage in competing with other bacteria. I thus consider that in only one percent of the cases will the foreign DNA be either beneficial to and/or no burden on the bacterial host.

In estimating the probability for exhibiting adverse consequences, I have considered that most eukaryotic DNA sequences will not be expressed in bacteria, and that only one out of 10,000 of the one million mammalian genes present in the mammalian genome, if expressed, would be harmful to some organism in the biosphere. Thus, only one out of one million surviving cloned DNA segments might actually display detrimental properties.

In the second example, the robust E. coli is used, with a nonconjugative pSC101 plasmid vector. All probabilities remain the same as for the first example, except the probability for transmission of recombinant DNA, which is now reduced to 10^{-19} , that is one with another 10 zeroes on this number, per surviving escaped bacterium per day.

In these first two examples, the potential problem is therefore associated with a reasonably high potential of the escaped bacteria to survive in nature.

In the last example an EK2 E. coli host is used, and since this strain should not be able to survive in nature, the perpetuation of the recombinant DNA in nature becomes equal to the probability of survival times the probability of transmissibility, an exceedingly unlikely event.

Lights, please.

It is therefore my current opinion that the use of genetically disarmed host-vectors in conjunction with appropriate physical containment facilities and practices should reduce the probabilities of danger, if indeed they exist at all, to an astronomically small value. Nevertheless, this is not a reason to reduce our guard, since much information is lacking, and information is needed to permit a more accurate assessment of these potential bio-hazards associated with recombinant DNA molecule research.

Thank you.

DR. FREDRICKSON: Thank you very much, Dr. Curtiss, especially the additional small abstract on those interesting strains which are numbered with some extraordinary historical perspective.

Drs. Singer, Berg, Hogness and Curtiss will be, I think, with us this afternoon. However, if there are any questions to any of these individuals, particularly that any member of the committee would like to bring up, we can afford to spend five or ten minutes in that exercise at this time.

Mr. Hutt?

MR. HUTT: Don, I am not sure whether they are the persons to address the question to, but one question that occurs to me is that we have not considered how easy these experiments are--that is, what type of equipment is necessary simply to do the type of scientific experimentation, how readily available the raw materials or whatever you call them are, and therefore whether these could be done, for example, by a high school biology teacher or by a college level, or only a graduate level, et cetera.

DR. FREDRICKSON: That is a fair question. Dr. Berg?

DR. BERG: Well, the ease of doing the experiments depends on what you want to achieve. I think if one were to ask can you make a recombinant DNA--mixture of recombinant DNA molecules, the answer is that you could probably do it in a few minutes if you sent out for the enzymes and got some DNA samples and stirred them up, and you could manufacture them.

The problem of isolating a single gene is a much more complex problem and requires a great deal more sophistication, both in the materials that you start with, the handling, the fractionation, and eventually identification.

So there are aspects of it which are simple. The joining together of two molecules of DNA is simple. The cloning and isolation of a specific gene is exceedingly difficult.

MR. HUTT: The cloning, at what level could that be done, Dr. Berg, when you say exceedingly difficult? Only in the most sophisticated graduate schools?

DR. BERG: I think only in the laboratories which have had a great deal of experience in molecular genetics, microbiology, and in the physical methods that are needed to characterize--

DR. MELNICK: But isn't it true that the dangerous experiments can be done very easily?

DR. BERG: It depends on how you define the word dangerous.

DR. MELNICK: The ones without the knowledge of what the outcome is going to be, or portends to be.

DR. BERG: That is, a shotgun experiment, which is just to mix DNA from a primate organism mixed with a potential vector, the cleavage of the two, the joining together in random association is exceedingly simple.

MR. HUTT: It could be done in a high school laboratory?

DR. BERG: I suspect so.

DR. MELNICK: And one could not, at this point, define the hazards?

DR. BERG: Well, the hazard involves introducing it into an organism, and that takes a little bit more sophistication, because I think just having a test tube full of random recombinants is, by itself, not much more than taking two different DNA samples and mixing them up in the test tube in the first place.

The key part of this is, of course, introducing it into a bacterium which can amplify and clone various segments, and thereby provide a vehicle for transmitting it to the outside. I think that is the hazard.

DR. FREDRICKSON: Mr. Ladwig has a question?

MR. LADWIG: In the guidelines there is not anything spelled out about what happens if somebody goes beyond the guidelines, and what if somebody gets really excited about what he is doing and goes past that, what has been outlined here. What kind of penalty clause is there going to be? Does he just lose his grant? Or is he getting involved in a criminal type area?

Was any thought given to that in these guidelines, or is that not a question for this board.

DR. FREDRICKSON: Dr. Stetten, would you or a member of the committee like to respond?

DR. STETTEN: I believe it reflects the committee's belief that any significant alteration in the protocol from that upon which the approval was ordered must be reported back to the study section and re-evaluated.

Of course, the NIH in general lacks an effective police arm, and precisely how this can be enforced, I don't know. The only sanction that I know of that the NIH can ever exert, is to withhold funds.

MR. LADWIG: But is that enough?

DR. STETTEN: It is all we have got.

DR. FREDRICKSON: I think, Dr. Stetten, that Mr. Ladwig's question is a very good one, and I think that at some point in our discussion we will describe a little bit further for you the nature of our guidelines and where they might go. I think this is a part of your question.

Mrs. Peterson?

MRS. PETERSON: That relates somewhat to my question a little bit. I guess it is in two parts. First, are there any formal restraints now for all of this kind of experimenting and investigation that we have seen, and then the second part of that which would relate to it is I don't know what the ballpark is, I guess I should say. Are all these experiments only under grants from the NIH, or are people free on the side to go and experiment whenever they want?

DR. FREDRICKSON: Dr. Jacobs?

DR. JACOBS: We are developing a procedure which is analogous, or a policy analogous to the one for the research on or involving human subjects, where whether or not NIH funds the research it must be done under the scrutiny of an Institutional Human Research Committee, and approved by that committee.

MRS. PETERSON: That might be a committee--

DR. JACOBS: Excuse me, one second. If there are any infractions, NIH would not only withhold funds if it were the granting agency, but even if the work were done under grants from some private organization NIH would exercise the sanction against the institution to make it ineligible for grants for other purposes. We would do the same thing here for the DNA recombinant. So in that way we have some strength in enforcing the guidelines, to the extent that we can be apprised of what is going on in those laboratories.

Now, there is one important point about all of this work, and that is that the only reason for doing the work is to make contributions to knowledge, and you make the contributions to knowledge by eventually publishing your results, and if, in the publication of the results you reveal that you have been doing things improperly, say, like working with a botulinus toxin gene when that is proscribed, you are subject to the criticism of your peers, and it doesn't do you any good to advertise yourself as a very irresponsible individual.

So there is a peer pressure, also, to see that the work is done within the constraints which the scientific community is developing.

DR. FREDRICKSON: Technically, then, Mrs. Peterson, we are discussing guidelines here to influence research that is supported by the NIH or in institutions where other support is given to that institution from NIH. Actually, though, I think it not an exaggeration to say that we are setting a precedent here in terms of setting guidelines that are very likely to be adopted by many other funding agencies within this country, and probably around the world. The nature of the guidelines, their legal standing, I think is again something that I think we ought to take up in a little more detail.

DR. BERG: May I add one point to that?

DR. FREDRICKSON: Yes, Dr. Berg.

DR. BERG: I think there are institutional responsibilities in fact, where most of the laboratories where this work will be done. By that I mean universities, research centers, and they in fact are looking to these guidelines to develop inhouse review which will be independent of the granting process.

In fact, Dr. Rosenblith, who is sitting right next to you is the chairman of the group at MIT that will, in fact, review such procedures, such experimental work at MIT. There are equivalent groups at Harvard, at Stanford, and I think at most major institutions where such work is likely to be undertaken, and in fact in some cases that review is going down to the departmental level, because in our own department at Stanford, we have a departmental review of any such procedures and any such experiments.

DR. ROSENBLITH: One little footnote. I am not the chairman; I appointed the chairman.

(Laughter.)

DR. FREDRICKSON: Dr. Berg, while you are there, Mrs. Peterson also asked another question which no one has answered, and that is under what groundrules are we proceeding today in recombinant DNA research?

DR. BERG: Well, I think they differ in different places. As I understand it, the NIH has already in fact an inhouse review committee which has been acting on experimental protocols generated here. I think by and large most people, at least in this country, are using the Asilomar guidelines, as crude and as vague as they were, until the permanently considered ones will be promulgated.

In other countries there are a variety of conditions. In Great Britain it is more stringently proscribed than it is here, at the moment; but in various countries, and I think there is a summary of these in your book, and in some cases it says there is absolutely no review and constraint. In Australia there is a committee which is reviewing proposal by proposal, and approving or disapproving them to carry on this work.

DR. FREDRICKSON: Dr. Hudson?

DR. HUDSON: We have been told that there are 27 designated high-containment centers with facilities available, with nine others under way. I wonder if there will be certain types of grants only provided for these 27 plus the other possible nine, and will this produce some types of problems for other areas where there might be competent personnel but without facilities of a high-containment nature?

DR. FREDRICKSON: Dr. Stetten?

DR. STETTEN: The committee early considered the mandate from the Director of NIH as to whether specific support was needed at this time for the construction of additional high-containment facilities. The Director of the Cancer Institute had indicated that he believed that he could provide funds for such if these were needed.

After we heard Dr. Barkley's report on the number already in existence and as interest in biological containment rose over the months, the committee, I think, expressed disinterest in the specific support of additional high-containment facilities as not a matter of primary requirement at this time, but there were enough to do what anybody at this time seemed to feel had to be done.

DR. HUDSON: Would this permit the movement of competent scientists from one area to another where these facilities do exist for this kind of effort?

DR. STETTEN: I think maybe Dr. Barkley could speak to that.

DR. BARKLEY: At the present time there does not appear to be an interest among scientists who would carry out P4 studies to relocate. There has been expressed an interest to do some risk assessment studies which could be done in a short period of time in containment facilities that might be available.

At the National Cancer Institute consideration is being given at this time to providing some space to the Frederick Cancer Research Center for such interim uses of facilities for hazard assessment studies.

DR. FREDRICKSON: Judge Bazelon?

JUDGE BAZELON: I would like to ask a question here. At the high school laboratory level, what are the chances of a major disaster? Is there anything that can happen at the level that they can operate at?

DR. FREDRICKSON: Dr. Berg, or Dr. Singer, or Dr. Hogness? Dr. Berg, do you want to try that?

DR. BERG: I think, again, it depends on the kind of experiment that is being done.

JUDGE BAZELON: Well, I am talking about the high school lab.

DR. BERG: Yes, I mean that. For one thing I think one should take into consideration that the exposure or publicity of this issue has already alerted high school teachers and students to potential hazards that might grow out of such experiments, so I don't think that it would be done in an atmosphere of complete ignorance.

But assuming they were, what kinds of experiments are they likely to do? They might take a frog's DNA and they might in fact take a bacterial culture of E. coli and a plasmid, and mix them, cut them, and they try to see if they could introduce it into that bacterial cell. It would be very difficult in a high school classroom, in fact, to assess or determine

whether that experiment has succeeded, because in fact one has to have some fairly sophisticated way of knowing whether the bacterium has taken up the plasmid and has taken up the new section of DNA, and that again requires--let us assume that they have mixed them and they have got something, and they then pour it down the drain.

MRS. PETERSON: But that means that the damage could have been done, doesn't it?

DR. BERG: That is quite likely, that if they had carried out this experiment and if one assumes that there is that type of risk, then that could be done.

Now, as I say, most likely the type of DNA that a high school class would use is not likely to be the DNA for botulinus, and it is not likely to be from an organism which we consider pathogenic, because they are already not available to those groups.

So it is only the lowest-risk types of organisms that one is likely to have involved in such experiments, and already in regard to risks they will know that the containment requirements and the types of vectors suggested are almost at the lowest level. So I don't think there is a finite chance, frankly, of any disaster coming out of that kind of an experiment.

MR. HUTT: You say those are not available. Is that absolutely certain, the dangerous kind, under any circumstances?

DR. BERG: The availability of some of those organisms, class three, four, and five organisms is proscribed to those groups.

DR. FREDRICKSON: Dr. Singer would like to supplement your answer.

DR. SINGER: I just wanted to say this, that this whole notion that these experiments that could be done in high school laboratories was raised very early on in a conversation to give some idea that the experiments were relatively simple to do. But I think in fact it has gotten somewhat overblown by now, because I think it is highly unlikely that such experiments would be undertaken in high school laboratories for a couple of reasons.

First, there is the reason that Dr. Berg already alluded to--that it is one thing to mix everything together, and another thing to know whether in fact you have done an experiment and have had something work. Doing that is more difficult, and I don't think by and large that people undertake experiments in high school laboratories that have no purpose. They are usually set up for the instruction of students, and mixing two solutions doesn't teach anybody anything.

In addition, there are certain components that are required to do the experiment which are not that readily accessible--which are expensive, in fact, particularly when you buy them. They are costly things to buy, and I tend to think that this notion that the experiment will go on in any way in high school laboratories has probably gotten blown up out of proportion.

DR. FREDRICKSON: Judge Bazelon?

JUDGE BAZELON: But if what they can do goes down the drain, then what?

DR. SINGER: What I am trying to say is that I really don't myself believe that this experiment would be undertaken in many, if any, high school laboratories, because there is no purpose to it unless you are in the position to determine whether the experiment is working or if you have learned anything.

Then, in addition, I think it is also true that as I pointed out before, the kinds of DNAs that would be available to high school students are not of the sort that one would consider the most dangerous.

But I think the thing I want to stress mostly is that this was an idea of a way to express the fact that the experiments were relatively simple, but I tend to think that it really has been put out of context.

DR. FREDRICKSON: We will resume, on this very question if you like, immediately after we convene from the luncheon break. This committee will reconvene at 1:30.

(Thereupon, at 12:35 p.m., a recess was taken until 1:30 p.m., the same day.)

AFTERNOON SESSION

1:40 p.m.

DR. FREDRICKSON: Dr. Barkley, are you ready? I am going to alter slightly my forecast at the close of the last session. I think it wiser that we begin with Dr. Barkley's abbreviated discussion of certain procedural and practical problems, the description of a review committee, and of the containment possibilities and the features of those facilities.

Then we will reopen committee discussion, but I am going to move forward the time of public statements to the committee from 4:00 to 3:00, because we now have nine individuals who have filed an intent of their desire to make such statements before the committee, and I want to be sure that we give ample time for their presentations which will be 10 minutes and not longer, and then we have at least five minutes for the committee to interact with each of those individuals if they so desire.

So Dr. Barkley, you have already been introduced to us as the expert on containment, and I believe your official title is Director, Office of Research Safety in the National Cancer Institute. We welcome your description of these facilities.

DR. BARKLEY: Thank you, sir. I will not discuss at this time the procedures used by the NIH Biohazards Committee for review of intramural research programs involving recombinant DNA molecules. To continue the discussion of this morning, I will go directly into a description of the physical levels of containment that have been recommended in the committee's report.

Safety guidelines for handling hazardous microorganisms have been recommended by a number of governmental agencies. The guidelines are consistent and each varies according to the actual risk potential of the agent handled. In general, the guidelines fall into three or four distinctive control categories. The objective of each category, however, is the same. That is, to reduce or eliminate hazards or health risks in the handling of hazardous agents by reducing or preventing exposures to that agent. The difference in the control categories is in the margin of safety that is provided in preventing exposures.

Dr. Singer stated that the committee has drawn heavily upon the experience of a number of people in formulating recommended physical control measures. Four principal sources have been used in the formulation. Most importantly, I think, is the booklet Classification of Etiologic Agents on the Basis of Hazard. This document which was prepared by the Center for Disease Control (CDC), PHS, DHEW, places microorganisms capable of inducing disease in man into four categories according to risk. Descriptions of physical containment necessary for the control of risks in each of the four categories are presented. Further description of guidelines are presented in the CDC "Lab Safety Manual."

The other two documents are guidelines written by the National Institutes of Health for biosafety in general and the National Cancer Institute for oncogenic viruses.

Before describing levels of physical containment, it is important to understand two points. First, data on laboratory-acquired infections, collected by Dr. Pike and the late Dr. Sulkin of the University of Texas, indicate that the causative event which results in laboratory-acquired infections is unknown in 80 percent of all reported laboratory infections. Second, most microbiological procedures have been shown to possess the potential for creating aerosols. An aerosol creates two problems. An organism suspended in air can gain entry into the respiratory tract of the laboratory worker through inhalation. Airborne organisms can also settle on work surfaces or objects and can be transferred to a site where an

infection can occur. This knowledge suggests that aerosols may play a major role in the causation of laboratory acquired infections.

Physical containment is primarily used to prevent the release of aerosols. Containment is dependent on technique, specially designed physical containment apparatus such as safety blenders, and biological safety cabinets. Technique can minimize, but not prevent, the release of aerosols. Containment apparatus and safety cabinets can be designed to reduce or eliminate exposures to aerosols released during the conduct of microbiological procedures.

The principal device for containing hazardous microorganisms is the biological safety cabinet. I will provide a description of biological safety cabinets and then discuss the various combinations of technique, cabinets, and facility features which comprise the four levels of physical containment (P1, P2, P3 and P4) proposed for recombinant DNA research.

Biological Safety Cabinets suitable for confining operations involving recombinant DNA molecules are classified as Class I, Class II, and Class III Cabinets.

The Class I cabinet is a ventilated cabinet for personnel protection only, with an unrecirculated inward flow of air away from the operator. The exhaust air from this cabinet may be filtered through a high-efficiency or high-efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is suitable for research work with the Center for Disease Control (CDC) classes of etiologic agents 1, 2, and 3 where no product protection is required. This cabinet may be used in three operational modes; (1) with an 8-inch high, full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves.

The Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA-filtered recirculated mass air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. Two models of this cabinet are available, Type 1 and Type 2. The Type 1 recirculates approximately 70 percent of the air. The exhaust air from this cabinet may discharge into the laboratory or be diverted out of the laboratory. This cabinet is suitable for CDC classes of etiologic agents 1, 2 and 3. Vapors or gases which are hazardous from a toxic, radioactive, or flammability standpoint should not be used in this cabinet because of the high quantity of recirculated air. The Type 2 cabinet recirculates approximately 30 percent of the air. The exhaust air from this cabinet is normally ducted out of the laboratory through a HEPA filter and, occasionally, an activated charcoal filter depending on the operation. The cabinet may be used with gases or vapors that are hazardous from a toxic, radioactive, or flammability

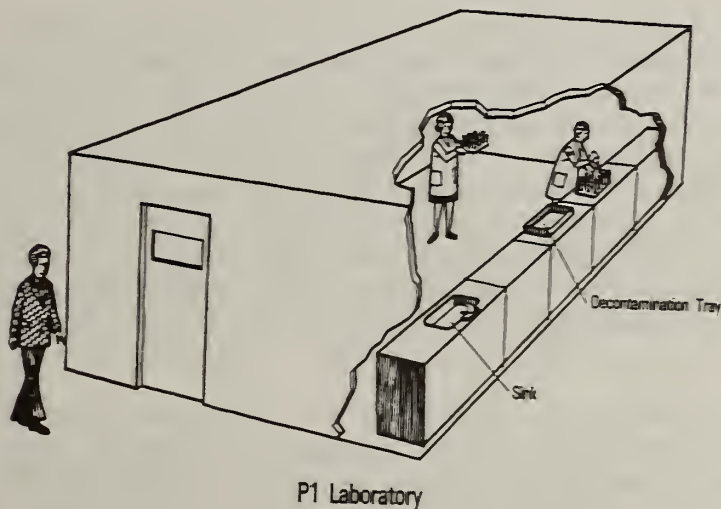
standpoint. However, any consideration of use of such materials should be evaluated carefully from the standpoint of build-up to dangerous levels and problems of decontamination of the cabinet.

The Class III cabinet is a closed-front ventilated cabinet of gastight construction providing total protection for personnel and product from contaminants exterior to the cabinet. The cabinet is operated under a negative pressure of at least 0.5 inches water-guage. All supply air is HEPA-filtered. Exhaust air is HEPA-filtered or incinerated to protect the environment. This cabinet, fitted with arm-length rubber gloves, provides the highest containment of these three classes of cabinets and is utilized for all activities involving high-risk agents (i.e., CDC etiologic agents, class 4).

Now I will discuss the four levels of physical containment described in the guidelines.

P1 Level (Minimal). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is a laboratory that possesses no special engineering design features (Figure 1).

Figure 1



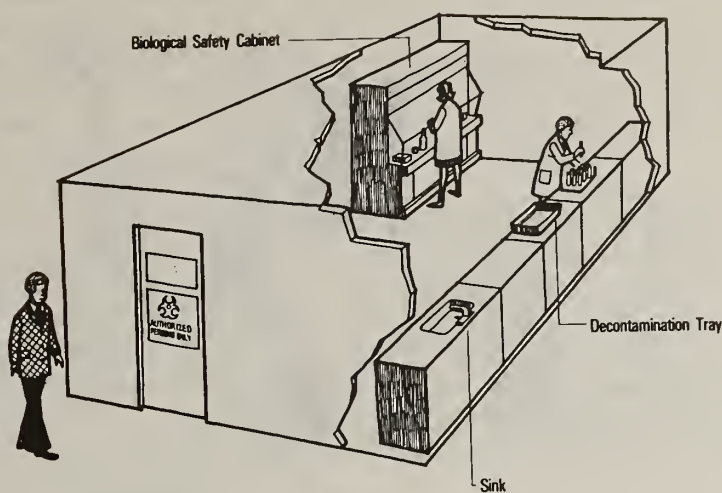
It is a laboratory commonly used for undergraduate-level teaching purposes and for other activities involving microorganisms of no or minimal biohazard under ordinary conditions of handling. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available in this laboratory. The laboratory is not separated from the general traffic patterns of the building. Public access is permitted.

Materials and equipment that contain or have come in contact with recombinant DNA materials are disinfected or sterilized by autoclaving prior to disposal, and work surfaces decontaminated. Cotton plugged pipettes and mouth pipetting may be used where the hazard is minimal, but mechanical pipetting devices are preferable and are required for more hazardous material. Pointed syringe needles are avoided where possible. Eating, drinking, and smoking are discouraged in the work area. Laboratory personnel should wash hands before and after experiments involving these materials. Laboratory doors are closed while such experiments are in progress. Appropriate clothing such as clean laboratory coats, or similar apparel, and closed shoes are worn when handling potentially hazardous organisms. Laboratory coats are not worn outside the work area. Each laboratory has an effective insect- and rodent-control program. Any research group working with agents with a known or a potential biohazard has an emergency plan, including a clean-up procedure to follow if an accident contaminates personnel or environment. The principal investigator ensures that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers are immunized. Serological monitoring, where appropriate, is provided for.

P2 Level (Low). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level is similar in construction and design to the P1 laboratory (Figure 2). The P2 laboratory has access to an autoclave, within the building; it may have a Biological Safety Cabinet. Work which does not produce a considerable aerosol is conducted on the open bench. Although this laboratory is not separated from the general traffic patterns of the building, access to the laboratory is limited when experiments requiring P2-level physical containment are being conducted. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

The P2 laboratory is typical of laboratories found in diagnostic facilities and college and university departments of microbiology. The laboratory is commonly used for experiments involving microorganisms or ordinary potential biohazard, such as those which have been classified by the Center for Disease Control as Class 2 agents.

Figure 2

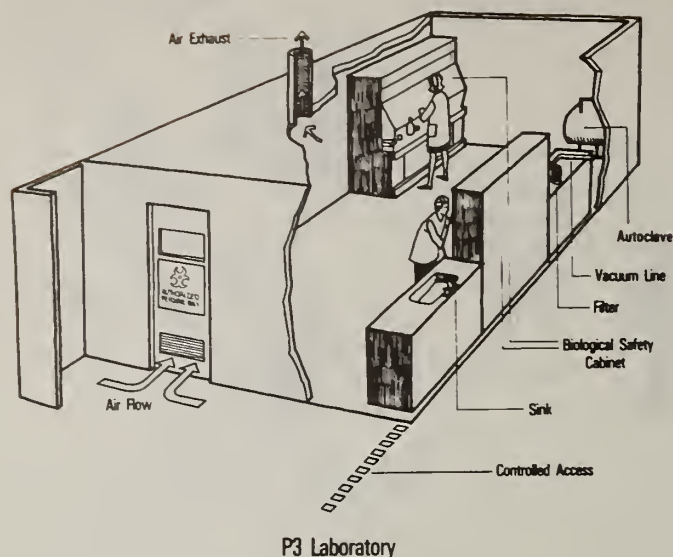


P2 Laboratory

The following safe microbiological practices and laboratory techniques in addition to those identified for P1 apply to all experiments requiring P2-level physical containment. Access to the laboratory is controlled when handling potentially hazardous organisms requiring P2 containment, but not otherwise. During the controlled period, appropriate biohazard signs are posted at access points to the laboratory, and only authorized persons who have been advised of the potential biohazard enter when such signs are posted. The signs are removed upon completion of decontamination after the hazardous procedures. Experiments of less biohazard can be carried out concurrently in carefully demarcated areas of the same laboratory if necessary, given that adequate precautions are taken to avoid cross-contamination and no animals or plants are in the area. Mouth pipetting is prohibited; mechanical pipetting devices are used. Specific precautions are required for those procedures that have a high potential for release of aerosols containing potentially hazardous material--e.g., centrifugation should be carried out in airtight cups or rotors, and sonication and blending are similarly contained or carried out in appropriate enclosed biological safety cabinets. All potentially hazardous material is inactivated before it enters the drainage system.

P3 Level (Moderate). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment (Figure 3). The laboratory is separated from the general traffic patterns of the building. Access to the laboratory is controlled. Biological Safety

Figure 3



Cabinets and an autoclave are available within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination.

Directional air flow is provided within the controlled laboratory area. The ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. The general exhaust air from the laboratory is discharged outdoors and so dispersed to the atmosphere as to prevent reentry into the building. No recirculation of the exhaust air shall be permitted without appropriate treatment.

No work in open vessels involving infectious hosts or vectors containing recombinant DNA molecules requiring P3 physical containment is conducted on the open bench. All such procedures are confined to Biological Safety Cabinets.

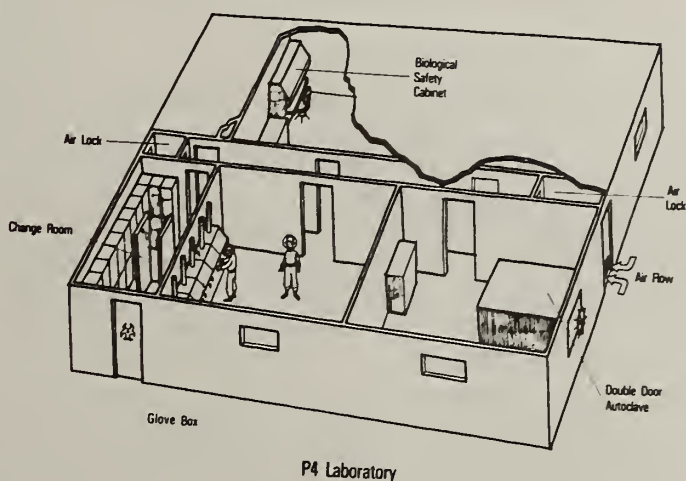
The following safe microbiological practices and laboratory techniques in addition to those identified for P1 and P2 apply to all experiments requiring P3-level physical containment. Appropriate biohazard signs are posted at access points to the laboratory, and only persons specifically authorized by the principal investigator enter when these signs are posted. Normally, authorized persons are limited to those who work in the laboratory.

Properly installed biological safety cabinets, meeting appropriate NIH performance standards are used for all transfer operations and for all procedures likely to produce aerosols. Operations that produce large amounts of aerosols, such as blending, sonication, vortex mixing, and vigorous agitation, are avoided where possible. Where necessary, they are carried out in enclosed systems that are not opened until adequate time has been allowed for aerosols to settle. Gloves are worn during the handling of biohazardous materials. Vacuum lines are protected by filters.

P3-level research can be conducted in laboratories where the room air flow and general exhaust air conditions described above cannot be achieved, provided that this work is contained in closed systems which are maintained under a negative pressure of at least 0.25 inches water-gauge. Operations including centrifugation, fractionation, sonication, etc., are contained in these systems. All materials, before removal from these systems, are sterilized or transferred to a sealed unbreakable container which is then removed from the system through a chemical decontamination tank, or after the entire system has been decontaminated.

P4 Level (High). Experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease (Figure 4). The

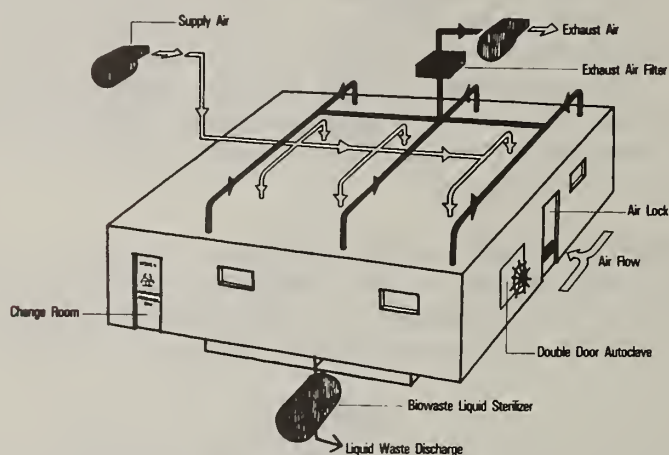
Figure 4



facility is isolated from general traffic patterns of the building. Access to the facility is under strict control. Class III Biological Safety Cabinets are available within work areas of the facility.

A P4 facility has engineering features which are designed to prevent the escape of microorganisms to the environment (Figure 5). These features

Figure 5



P4 Laboratory Suite
Secondary Barriers

include: (1) monolithic walls, floors, and ceilings in which all penetrations such as for air ducts, electrical conduits and utility pipes are sealed to assure the physical isolation of the work area and to facilitate housekeeping and space decontamination; (2) air locks through which supplies and materials can be safely brought into the facility; (3) contiguous clothing change and shower rooms through which personnel enter into and exit from the facility; (4) double-door autoclaves to sterilize and safely remove wastes and other materials from the facility; (5) a biowaste treatment system to sterilize liquid effluents if facility drains are installed; (6) a separate ventilation system which maintains negative air pressures and directional air flow within the facility; and (7) a treatment system to decontaminate exhaust air before it is dispersed to the atmosphere. A central vacuum utility system is not encouraged; if one is installed, each branch line leading to a laboratory is protected by a high-efficiency particulate air filter.

The following safe microbiological practices and laboratory techniques apply to all experiments requiring P4 level physical containment:

- a) The universal Biohazard sign is posted on all facility access doors and all interior doors to individual laboratory rooms where experiments are conducted. Only persons whose entry into the facility or individual laboratory rooms is required on the basis of program or support needs are authorized to enter. Such persons are advised of the potential biohazards and instructed as to the appropriate safeguards to insure their safety before entry.
- b) Personnel enter into and exit from the facility only through the clothing change and shower rooms. Personnel shower at each exit from the facility. The air locks are not used for personnel entry or exit except for emergencies.
- c) Street clothing is removed in the outer facility side of the clothing change area and kept there. Complete laboratory clothing, including undergarments, pants, and shirts or jumpsuits, shoes, head cover, and gloves, are provided and used by persons who enter the facility. Upon exit, this clothing is discarded into collection hampers before personnel enter the shower area.
- d) Supplies and materials to be taken into the facility are placed in an entry air lock. After the outer door (opening to the corridor outside of facility) has been secured, personnel occupying the facility retrieve the supplies and materials by opening the interior air lock door. This door is secured after supplies and materials are brought into the facility.
- e) Doors to laboratory rooms within the facility are kept closed while experiments are in progress.
- f) Experimental procedures requiring P4-level physical containment are confined to Class III Biological Safety Cabinets. All materials, before removal from these cabinets, are sterilized or transferred to a sealed durable container, which is then removed from the system through a chemical decontamination tank, autoclave, ultraviolet air lock, or after the entire system has been decontaminated.
- g) No materials are removed from the facility unless they have been sterilized. All wastes from the Class II Biological Safety Cabinets and other materials to be reused (e.g., soiled laboratory clothing), discarded (e.g., trash), or removed from the facility are sterilized in the double-door autoclave.

Materials which may be damaged by temperature or steam are sterilized by gaseous or vapor methods in an air lock designed for this purpose.

- h) Eating, drinking, smoking, and storage of food are not permitted in the facility. Water fountains located in the facility corridors are permitted. Separate potable water pipes are provided for these fountains.
- i) Facilities to wash hands are available within the facility, and personnel are instructed to wash hands after experiments.
- j) An insect and rodent control program is provided.
- k) Animals not related to the experiment are not permitted in the facility.
- l) If an appropriate central vacuum system is provided, each vacuum outlet is protected by filters and liquid traps.
- m) Use of the hypodermic needle and syringe is avoided whenever possible.

I want to close on two points. First, I want to underscore again the importance of training. Workers in this field must be proficient in microbiological techniques--techniques that will prevent the release of contaminants from the experimental system. They must be proficient in standard safety practices. One does not confine a poorly contained study into a hood and assume that the hood is going to provide the protection. The technique is very important in preventing the release of these materials.

Second, everyone who occupies laboratory space where DNA recombinant studies will be undertaken must be knowledgeable of the risks associated with this work and the means by which this risk is to be controlled.

DR. FREDRICKSON: Thank you, Dr. Barkley.

I think that we will offer this opportunity now for members of the committee to question Dr. Barkley or to comment on the material that he has just described, if you would.

Dr. Petersdorf?

DR. PETERSDORF: Well, I wanted to make a comment which pertains to some of this morning's discussion to try to put into perspective some of the area of microbiological research with pathogenic organisms.

In doing so, I am not trying to minimize the importance of what is being discussed. Obviously, if an E. coli is inoculated with botulinus or diphtheria toxin, a great disaster may eventuate. But as a clinical investigator who has worked with pathogenic microorganisms for 25 years, I think it is important for the lay audience to appreciate that research with potentially hazardous organisms is going on all the time, and it is going on primarily to understand better at a clinical level the pathogenesis of infection, with attempts to prevent it or to cure it.

The fact of the matter is that the human host is a remarkably hardy biological being, and that with all of the pathogens about, we happen to survive remarkably well. In my own experience, for example, having worked in the 1950s with virulent Staphylococci, which at that time were causing major disease, having worked subsequently with Streptococci, with both the gonococcus and the meningococcus, which happens to cause infectious meningitis and which is a somewhat hazardous organism, and having worked a great deal with E. coli, I am not aware that nationally or locally in our own operation that we had a single laboratory accident among either the investigators or all of the ancillary personnel that worked in the laboratory.

I might say, without again minimizing what Dr. Barkley said, that these were all done in Pl laboratories, and I wish that the investigators had observed the conditions prescribed, because if I had a buck for everybody who pipettes while smoking cigarettes, I would be a very wealthy man.

Now, let us talk a little bit about E. coli, because this is the organism which has come to the fore this morning. All of us have somewhere between 10^8 and 10^9 of E. coli per gram of feces in our bowels. In the normal human host E. coli causes remarkably little disease. It is a non-infective and non-invasive organism, and it causes infection primarily in individuals who have decreased host resistance and who have structural abnormalities where E. coli can gain entrance. For example, in males it rarely causes infections in the kidneys unless a catheter is put in and the organisms have the opportunity to get up into the urinary tract or sit in the bladder, and to multiply there. In most of those instances the illness they cause is quite benign and in very few is the illness serious.

Thus, most of us, despite the fact that we exist with these organisms, this is not the strain which has been talked about here which is really quite attenuated. It causes us remarkably little harm, and only recently, for example, have a few strains been described that may cause an illness akin to traveler's diarrhea, but even that isn't as well established as perhaps it should be.

The other thing we ought to know about E. coli is that E. coli has, in fact, in nature undergone the process that we talked about today, the

introduction of a recombinant DNA on R factor, which renders certain strains of E. coli resistant to certain antibiotics.

Now, this has posed a few clinical problems, but not to the extent that was perhaps envisioned when this discovery was first made at least a decade ago. I don't think we can ignore the rather low-key statement that Dr. Hogness made, that he has literally worked with tens of thousands of E. coli into which recombinant DNA has been introduced without having any difficulty.

Indeed, the risk of laboratory accidents that I see or that I hear about are much more likely to be with chemicals or the physical means of laboratory techniques than with microbiological techniques. And indeed, if one follows even some of the excellent procedures outlined by Dr. Barkley, much of the hazard that we envision from these organisms will not be apparent.

Finally, I just want to say a word about the risk of this happening in high school laboratories. My teenage son goes to a very high-class, I might say, expensive and sophisticated high school, and while he knows about recombinant DNA--as a matter of fact they do teach this material in his senior biology course--experimentally they haven't progressed much above the level of making cheese from goat's milk, which is very dangerous to the olfactory system, but otherwise quite a simple procedure.

So I think that the point that has been made--that the technology in high schools, to have this a regular exercise, is available--it simply isn't, for no other reason than it is too expensive for the average high school to do it.

I think the things that are going on here are terribly important, but I simply wanted to make these remarks as one who has worked in the field of clinical microbiology and infectious disease for 25 years, and who has survived it reasonably well, and to point out to you that we are all pretty well constituted, and that the hazards are the potential hazards of what we are talking about, and they may not be as great because the good Lord made us what we are.

MR. HUTT: I have one specific question without responding, but I would like to return to the questions that have been raised.

The specific question is how many P3 facilities are there in the country? We heard there were 27 P4s, but we have not heard anything about the number of P3 facilities.

DR. FREDRICKSON: Dr. Barkley?

DR. BARKLEY: I can't give you an answer to that. I would think that most of the facilities constructed over the last two decades have incorporated into them the principal means which would allow them to be--or the laboratories within those facilities--to be qualified as a P3 laboratory.

MR. HUTT: Do you have any dollar figure on what it would take to upgrade a P1 or P2 to a P3?

DR. BARKLEY: We are probably talking in the neighborhood of \$200 to \$300 per square foot.

MR. HUTT: But on P3 are you talking of there being 100 or 1,000 or 10,000 of them, Dr. Berg?

DR. BERG: I can just make the comment that almost anybody working with tumor viruses today has the equivalent of a P3 laboratory. Those are quite extensively located, one would say certainly in the hundreds.

But since I did build a P3 facility--convert a P1 to a P3--I can tell you it is not ... it costs, to convert a 400-square-foot space from a standard open lab to one which qualifies, I believe, as a P3 facility, about \$25,000, exclusive of any of the standard laboratory equipment, which would ordinarily be used. We had to supply this for this room so that we wouldn't use the departmental facilities. To make 600 square feet of space, it cost us altogether between \$35,000 and \$50,000 to convert that space into a high, P3 facility.

MR. HUTT: Well, it is very difficult to evaluate how much of a restriction P3 is over P1 and P2 without knowing how many laboratories there are that would meet that requirement.

DR. PETERSDORF: Most large institutions have at least one, I would say, and often several.

DR. MELNICK: I would say every medical school in the country must have at least one, if not more.

MR. HUTT: So that would mean that if you were to have a high level of requirement of P3 and eliminate some of the lower level P1 and P2 it would not be that big a restriction on research?

DR. FREDRICKSON: Dr. Hogness?

DR. HOGNESS: I think that if you are doing this kind of research day in and day out and you have one of these facilities at the University of Washington or something, that is not going to be anywhere near enough. This

is an expanding field, and the existing facilities are now used for other purposes. You would have to increase production quite a lot if everything went to P3 in this area.

DR. PETERSDORF: But the conversion isn't very expensive, or very difficult. That is the point, I think, that Paul made.

DR. HANDLER: Don, may I ask a question?

DR. FREDRICKSON: Yes.

DR. HANDLER: I don't think the capitalization cost of building these things is what the problem is. It seems to me the question is how much more difficult does it make doing the research, how much more time was consumed, how many more people do you have to have, how much more time does it take to do an experiment after you have invested what capital cost? If it doesn't, then I think it is an empty discussion.

DR. FREDRICKSON: Dr. Berg, did you want to say more on that same issue?

DR. BERG: Well, I think there is no doubt that the implementation of these guidelines is going to make research go more slowly. I doubt that there is anybody here who thinks he can move any other way as rapidly or as efficiently under these types of guidelines as he could before.

MR. HUTT: But if you were to require a greater use of P3, certainly the concern about doing things in high school laboratories would be substantially reduced because not many high school laboratories would have a P3 facility.

DR. KELLY: I thought the Judge's question was whether or not they would be done under conditions that were not safety-oriented.

DR. FREDRICKSON: Dr. Zaitlin?

DR. ZAITLIN: I would like to just point out what I consider to be one technical defect in the recommendations for certain types of experiments in which plant DNA viruses are used as a vehicle to introduce the foreign DNA material into the plant.

The conditions specify that we should use the best insect-proof greenhouses and keep them under negative pressure. I wish to point out that these plant viruses are normally transmitted by insects, and these sorts of procedures would encourage bringing those insects into the greenhouse rather than having to keep the vectors out. I think this is a serious defect in your recommendations.

DR. FREDRICKSON: Dr. Walters?

DR. WALTERS: Dr. Barkley, you mentioned statistics that have been kept on laboratory-acquired infections. I wonder if you would mention what the number of documented cases of laboratory-acquired infections has been for perhaps the past 30 years.

DR. BARKLEY: As I pointed out, the late Dr. Sulkin and Dr. Robert Pike of the University of Texas have, for the last three decades been involved in collecting information from the literature and from personal contacts on the occurrences of laboratory-acquired infections. They have accumulated information on 3,921 cases; about 300 were reported earlier than 1925. The bulk of the 3,921 laboratory-acquired infections have been in the last three decades.

It is of interest to note that the number of recorded infections is probably a very low figure -- in fact, an extremely low figure. The numbers do not include subclinical infections. They only include those infections brought to the attention of Sulkin and Pike.

The other thing I would like to point out is that of the 3,921 cases over 900 have been involved in 35 micro-epidemics within laboratories. A micro-epidemic is one in which one exposure incident resulted in more than one infection among laboratory workers. Approximately 300 laboratory-acquired infections have been associated with the causation agent of Q fever, a very resistant organism.

I would like to point out that even though we have had 35 micro-epidemics resulting in over 900 individual infections, there have only been 43 infections of persons not directly associated with the laboratory. Twenty-nine of these infections were among personnel working in an animal rendering plant where hamsters infected with Q fever were sent.

No laboratory-associated infections, however, have been reported to our knowledge that have been among persons who had no association with the laboratory activity.

I think the other important thing to point out here is that the majority of these laboratory-acquired infections occurred in facilities where no biological safety controls were actually in effect.

The experience in facilities with upgraded safety measures, however, indicate an encouraging record of few laboratory-acquired infections. Physical containment can, therefore, be effective in controlling such infections.

DR. FREDRICKSON: I am not sure how much weight to put on the evidence you have just discussed, Dr. Barkley. There are no denominators, and as you yourself say--in fact, I would like to ask Dr. Walters what conclusions he draws from Dr. Barkley's answer.

DR. WALTERS: I think it is difficult to draw any conclusions, as you say, because there are no denominators, however it seems to be perhaps the only analogous area from the past in which there are any statistics that one can draw upon.

DR. FREDRICKSON: You would conclude that in some laboratories, infections may result? I think that would be a reasonable conclusion, but that would be the extent of it.

Dr. Melnick?

DR. MELNICK: Yes, I would like to emphasize what Dr. Petersdorf said just a few moments ago, and also Dr. Barkely emphasized the same thing. More important, it seems to me, than the physical equipment is the training that the person has working in a laboratory with infectious agents. I would like to recall a recent bit of information that our laboratory, for instance made together with Dr. Lennette's laboratory in Berkeley, California.

There was a virus that swept through Asia and Africa two years ago that infected at least 50 million people with a disease that caused hemorrhagic conjunctivitis, red eyes, and it spread over these two continents in a matter of a year. Our laboratory and Dr. Lennette's laboratory, concerned with identifying new viruses wherever they appear in the world--these viruses were sent to us. Both laboratories carried out work with the virus and identified this as a new enterovirus now called number 70.

DR. FREDRICKSON: Could you speak a little louder, please?

DR. MELNICK: Yes. These viruses were identified in both laboratories as a new virus which had not appeared in the world before, now called enterovirus number 70, and had this virus come before a group of this sort as to whether a laboratory should work on it in this country, I think the answer would be no. However, the virus has been identified and characterized. There has not been a single laboratory infection, either in Dr. Lennette's laboratory, where this was carried out, or in our own laboratories. There was no antibody conversation in the laboratory.

Now, the type of equipment where this laboratory work is done would be called, let us say, P1 or P2. My concern, where we get into laboratories of P4 variety is that the equipment is so tedious and so difficult

to work with in these laboratories, there are apt to be more spills than if one is able to work with greater ease, even in a P1 laboratory.

I would like in this regard to ask Dr. Barkley, who tells us there are at least 27 P4 labs around the country, how many of these are really functioning so that there is the absolute control that they are potentially capable of performing?

DR. BARKELY: To my knowledge at this time, I don't know of one of the laboratories that is currently being operated as a P4 operation; that is, that a full-clothing change is being carried out or all the materials are isolated. I don't think that the Frederick Cancer Research Center at this time is operating at the P4 level.

DR. MELNICK: What is the point then of trying to raise our level to, say, P4 containment if it is so difficult to work in a P4 unit that the containment that it should carry or be capable of performing is not being carried out?

DR. FREDRICKSON: Do you want to respond to that, Dr. Barkley, or Dr. Berg?

DR. BARKLEY: I would just like to say that there are a number of agents that require P4 control, and if it were of interest to conduct studies on them they would be placed in P4 facilities. I do know that at the Center for Disease Control they plan to initiate new studies on the Lassa virus. Now, that work will be confined to a P4 glove-box operation totally, and it would not be considered to work under conditions less than that. So it really depends on whether people are doing research that requires this.

DR. FREDRICKSON: What you are saying then, Dr. Barkley, is that these facilities exist and relatively moderate procedural changes would be required to get them into operation at the P4 level. Is that correct?

DR. BARKLEY: Yes, that is correct.

DR. FREDRICKSON: Dr. Berg, you have a comment on that?

DR. BERG: I would like to comment on two issues that were raised by Dr. Petersdorf and Dr. Melnick.

I think Dr. Petersdorf is quite correct in pointing out that E. coli is a very low-degree pathogen, and I think the fact is that biochemists and molecular biologists have grown up enjoying the use of them primarily and have become so familiar with them because they don't worry about dangers. I think we are not worried about E. coli infecting people. We are

worrying about what E. coli is carrying that we put in, and that is a very different issue, first of all.

Second of all I think we are talking about a difference in scale in the operation between what molecular biologists and biochemists want to do compared to what you have been doing over the last 25 years. You deal with them as small cultures, either in diagnostic work or for studies on a small scale. We want to be able to grow them in large quantities and isolate the molecules they carry, and I believe it is the scale which raises the questions about risk more so than the level you have talked about.

The third point, which I have only alluded to, is that we are not talking about individuals who are trained medical microbiologists or people who have grown with an experience and background in dealing with pathogenic microorganisms. I think we all recognize that if we were dealing with only those people we would only have to call to their attention the potential risk of certain experiments, and they would be well-enough trained to handle it.

When we drafted the initial report to the Academy, we recognized that the enormous influx of people into this field without that background, without that training and without that experience, but with this great familiarity, an almost contemptible familiarity with E. coli as being an innocuous organism. I think the issue we are raising now is that E. coli carries something. It is not E. coli, it is what it carries. And two, we are dealing with it on a scale which is different than has been done before; and three, it is being worked on by people who have not had the training or the background. Hopefully these guidelines will now force people to acquire that training and to use the best available techniques that you people have worked out over the last 25 years.

DR. PETERSDORF: But you know, the R factors that the E. coli carry hasn't done anything to its pathogenicity, even in man. So you are talking really about potential hazards and the speculation has to be. Now, I won't address myself to the scale situation which I can't comment on, and to the training thing which I agree with you on. The real speculation is what is the potential hazard? And just because E. coli carries something, does that enhance its pathogenicity? For that you have no evidence, as far as I can tell.

DR. BERG: We agree, then.

DR. FREDRICKSON: I think Dr. Curtiss wants to respond to this very same point.

DR. CURTISS: I would just like to add on to what Dr. Berg has said. I agree with him, but there are some additional things.

I don't think it makes so much difference that E. coli K12 carries this material. The fact is that normal wild-type E. coli K12, under optimal laboratory conditions, is capable of exchanging plasmid DNA with strains from over 40 genera of bacteria, including Salmonella, Vibrio, Pseudomonas, many of which are pathogenic.

The second point I would make is that I think we altogether place too much emphasis on the damage that these recombinants could do to humans. We don't happen to be an indispensable species in the biosphere. Plants and other microbes and things are, and we depend on them. I think we have to broaden our thinking of what organisms might be at risk. Although we like to protect ourselves, I think we have to consider the others.

DR. FREDRICKSON: Dr. Callahan?

DR. CALLAHAN: It seems to me the nature of this kind of discussion is rather confusing. I am not sure whether because there are potential benefits which can ensue from this research, the burden of proof is then on those who would like to point our harms, or vice versa, namely that the burden of proof is on people to suggest harms, and those who suggest benefits must then prove their case.

I think this is rather important, because as I listen to particularly the last exchange between Drs. Berg and Petersdorf, I worry about the problem of any discussion on a risk benefit ratio. As I listened to the discussion this morning, I got a sense that we are totally ignorant about the possibilities of whether all the potentialities will in fact eventuate. We are also equally ignorant about all of the harms.

Well, if we are really that ignorant in both directions, then it seems to me we have to perhaps work on the premise that the harms are as likely to eventuate as the benefits. Then if that is the case, I am very confused knowing how to listen to a discussion about safety features, because it seems to me that if the harms are as likely to eventuate, even if there is only a remote chance of things getting out of the lab, but should they get out we are going to have a disaster on our hands. That could be terribly influential in deciding whether the benefits are worth it or not.

I am at least left with the conundrum on my hands, and I suppose the problem goes back to: "Are we in fact equally ignorant of benefits in this case as of harms, or vice versa?" Again, let me put it that way.

DR. FREDRICKSON: Dr. Hogness?

DR. HOGNESS: I think perhaps you have missed one point that I made, and that is that there are certain benefits that we are talking of at all.

DR. CALLAHAN: Of knowledge, right.

DR. HOGNESS: Yes, well, you think the more you know the more you can cure, and that is an enormous benefit. It is also a benefit to the spirit, or to my spirit.

(Laughter.)

I think the other point that I would make in regard to our initial worry about these things and the possibility of epidemic, because that is what you are talking about--a catastrophe. If you are talking about some people getting sick, that is very bad, but if it is a few people, you can compare that with freeway accidents and lots of other things, but if it is a catastrophe, then we are talking about epidemics.

Now, the only facts we have on this argue against it, because when you take all the genomes from the yeast and put it into independently cloned lambda vector particles, we have thousands of them, but independent ones, so you are representing the whole yeast genome, the whole eukaryotic genome. You put them all into a pot, into a flask, and then put in one original natural lambda vector at the same level that each one of these is, so you have got 20,000 there and you add this one lambda, then they would successively infect bacteria. Then look after two generations. The only thing that is left is the original parent.

So in that sense and in that test they fared very, very poorly. That is, they were lost in this natural environment. That kind of thing makes us less worried about the epidemic, but not totally. Of course, you can always argue that there is some niche that some one of these might have taken over.

DR. FREDRICKSON: Dr. Singer?

DR. SINGER: Just to answer the question that Dr. Callahan raised, I would like to say that in a sense the guidelines have recognized that exactly the quandary that you raised, and have responded to it by saying that in those instances where the possible hazardous events would be of such an enormous magnitude, we therefore conclude for those experiments that they ought not be done; whereas those experiments for which there are specific recommendations--that therefore come under a class of things which can be done--are other than those which give rise to fears of massive disasters, or even modest disasters. So perhaps in a way responds to that.

DR. FREDRICKSON: Dr. Kelly?

DR. KELLY: I know so little about this that I ought to keep my mouth shut, but I thought that Dr. Petersdorf sort of sharpened an issue for us,

which is a policy issue that I would like to discuss later. But I thought that he identified the extent to which in the natural environment there was a tremendous capability of the body to resist invasion of that which was found in the natural environment. He has indicated the extent to which he has been engaged in the research which was not serious or dangerous. I would sort of like him to comment on the extent to which he is concerned about creating an organism that is not found in the natural environment and whether or not you can place the same degree of confidence in the body's ability to react in a manner which is not adversely affected by that organism which is not normally found in the environment.

He has identified the extent to which the safety precautions had been completely adequate where you were dealing with an organism that was found in the environment. What we are talking about is creating an organism that is not normally found in the environment, and what the degree of threat that that represents, and how much of a safeguard you should take against that.

DR. FREDRICKSON: Dr. Petersdorf?

DR. PETERSDORF: Well, I would like to share the answer with Dr. Melnick, who is a distinguished virologist, and I think in the virus field the answer may be somewhat different than in the bacterial field.

Dr. Curtiss certainly pointed out that some enteropathogens can be introduced into that parent bacteria which might potentially make them pathogenic. Now, there are two things I would say about that. One is where you know that, you don't do that experiment in terms of the--or you try not to do that experiment because that is one of the things which will be ecologically, I think, unsafe and potentially hazardous.

Furthermore, even when working with enteropathic organisms, such as Salmonella and Pseudomonas and some of the other ones, the incidence of infections in the laboratory, short of drinking a culture flask of that stuff, which very few of us would enjoy, is very small--in other words, the physical precautions within the knowledge that we have should take care of this matter.

Now, what we don't know, and where I agree that Dr. Berg has a point, we may be creating something somewhere along the line, a potential pathogenicity of which we do not know, and the response of the host we do not know. I think this is probably the biggest danger.

I have been impressed listening to the molecular biology of this meeting, that the E. coli, as long as you use that as a host, has been attenuated, so I am not at all sure that you are ever going to do a hell of a lot for that particular E. coli to make it hazardous for man. I can't comment on what it might do to other organisms in the environment because

I simply don't know. I think there are somewhat different considerations in the virus field, and perhaps Dr. Melnick would like to comment on those.

DR. FREDRICKSON: Dr. Melnick?

DR. MELNICK: As I have listened to the sessions today, there is a big gap in knowledge as to whether the recombinant DNA is expressing itself in E. coli. The only experiments that I heard about this morning were the ones that Dr. Hogness did, where he put Drosophila DNA into E. coli. I wonder if he could tell us a little bit about the expression of that DNA.

DR. HOGNESS: There is one experiment in which you look at random inserts of DNA into plasmids in the following way. There are bacteria which generate mini-cells which don't contain the E. coli chromosome, but they do contain the plasmids. So you can ask what proteins are made from the genes in the plasmid without being bothered with all the background that is generated by the chromosome of the E. coli.

When 50 hybrid plasmids were looked at in this way, five new proteins were detected which could have come from the inserted Drosophila DNA. Since each hybrid contains 10,000 base pairs on the average, a total of 500,000 base pairs was looked at. Hence, we detected only one new protein per 100,000 base pairs of Drosophila DNA. This is a maximum estimate.

DR. MELNICK: Are those proteins Drosophila?

DR. HOGNESS: We don't even know that those five were coded by Drosophila sequences. They could have been. By comparison, when the same experiment was done with inserted DNA from a prokaryote, B. subtilus, an average of one new protein per 1,000 base pairs of B. subtilus DNA was detected. Hence, we conclude that expression from prokaryotic DNA is much more likely in E. coli K12 than expression from eukaryotic DNA, such as Drosophila.

DR. MELNICK: Are those proteins the same type that are expressed in Drosophila?

DR. HOGNESS: We don't know about that. All we know is that they are made due to the inserted Drosophila DNA. They could be due to a coding sequence within the parent plasmid, or to some disruption of the control mechanism. There are a lot of outside guesses for the mechanism by which these new proteins might have been produced.

There are similar experiments done with yeast by John Carbon, where you ask for a natural transformation of E. coli function, and then ask how frequent that is compared to the same thing when you are transforming with coli to coli. There again, the frequency is reduced for the eukaryote, yeast.

DR. FREDRICKSON: I just want to be sure that Dr. Kelly's question is answered well for those on the committee who may also have the same question. I think his question is that it is not then changing the infectivity or infectiousness of E. coli, but that we would be introducing through them chimeric forms of new genetic material, right, Dr. Berg? Let us be sure that there is no question about that.

DR. SINSHEIMER: May I speak to that?

DR. FREDRICKSON: Yes, Dr. Sinsheimer?

DR. SINSHEIMER: I have no technical information to contribute, but I share the concern that Dr. Petersdorf, who knows much better than I, has pointed out, that we are pretty resistant to most organisms, most likely by virtue of selection. Indeed, we all know what has happened to the Hawaiian Islanders, et cetera, when they were exposed to organisms novel to them. They were decimated.

The possibility of generating novel pathogens through the introduction of eukaryotic genes into prokaryotes, I think, cannot be neglected. We know, for example, that there are many viruses indigenous to prokaryotes which ordinarily are of no consequence to us or other eukaryotes because they lack, for example, the means for expression, the proper control sequences.

I don't see how we can know at this time that they couldn't acquire such control sequences from these chimeric organisms, and thereby become a potential hazard.

DR. PETERSDORF: But where virgin populations have been studied, that really have been decimated by new organisms, they have been almost entirely viruses. I think that only the totally germ-free pigs that are being produced are decimated by bacterial infections. Sure, there are bacterial epidemics, but I am not at all sure that in the recent past this has been a major problem with the populations in Hawaii or in Alaska among the Eskimos, where the problem has been almost entirely due to viruses. I think Dr. Melnick again knows more about that.

DR. FREDRICKSON: Mr. Hutt?

MR. HUTT: We come back to the question again of the burden of proof. I think this is fairly critical. I agree with Dr. Callahan, that the public is quite clear that far from being a burden on them to establish that there is a hazard, they look at it that it is the burden on the field of science that wants to proceed in these inquiries that there is no hazard. Until that burden is satisfied one way or another, with either the physical or biological containment concepts that we talked about today, I don't think the public will feel very good about your statements or any expert's

statements that it is unlikely that there will be a disaster, because any likelihood is not going to affect just you or your laboratory workers. We are talking about a possibility that would effect large populations and members of the public.

In addition, looking at the physical side, I will confess that my years of experience as a regulator leads me to conclude that there is no such thing as a fail-safe operation, a zero-defect operation, and if anything can go wrong, I have seen it go wrong, all over the place and all the time--so much so, indeed, that in many instances no one involved can ever explain afterwards what did go wrong.

There are many cases where you simply cannot tell where for example, an industry in the production of drugs or foods, there will be disasters that no one can explain. I anticipate that even under a P4 that would occur on occasion, and certainly under something as low as P1 and P2, I would have to assume that it would happen all the time.

Then you look at the question of the biological containment, and the very fact, Dr. Petersdorf, that leads you to conclude that this is relatively safe to use E. coli, leads the Boston Group to conclude that it is quite the wrong thing to use--namely, that if you have that level of E. coli that can reside in humans, then it is a very poor thing to use because it will easily be found in humans, and could carry these dangerous forms.

Now, I would like to hear some discussion of the cost in terms of lost scientific endeavor, in terms of money and time, in pursuing the concept that there would be either no E. coli form used at all, or that in any event it be at the bare minimum, the EK2 or EK3, which would then have the closest thing to a fail-safe system, certainly much closer than use of the physical containment.

DR. FREDRICKSON: Dr. Curtiss, would you like to take a whack at that?

DR. CURTISS: Not the cost part.

MR. HUTT: Well, what would it do? Would it destroy ongoing experiments? Would it preclude some type of new knowledge being gained in the future?

DR. CURTISS: Well, I think the point has been raised already, if we were to say okay, let us cancel E. coli and go to another system, I don't know that anybody has made even a suggestion as to what the other system might be.

The point is that, to my way of thinking, you can think of many microorganisms that are out there, in the soil or wherever, but the point

is that they have not been studied, and to assume because they have not been studied that they are safe, to me seems to be sort of an absurdity.

I mean, E. coli has been studied for years and years at millions and maybe billions of dollars of research money throughout the world, and all of the problems we are very familiar with.

MR. HUTT: Well, what about going to a requirement of no EK1, just EK2?

DR. CURTISS: Well, that would, I think, in terms of the probabilities I gave this morning. . . . If you use an EK1 system, it is very likely that that organism if released in abundance would survive and perpetuate the genetic information. So I would go along with that, I guess. I think that would be a reasonable ultimate goal.

MR. HUTT: But what would that result in, and perhaps I should ask Dr. Berg and Dr. Hogness, what would it result in in terms of lost time and effort and money, and scientific information if that requirement were imposed?

DR. FREDRICKSON: Dr. Hogness?

DR. HOGNESS: First, there are no EK2's at the moment.

MR. HUTT: I understand that.

DR. HOGNESS: So we already have some time loss.

MR. HUTT: Correct.

DR. HOGNESS: Second, I think the question really asks are there experiments you can only do with EK1 that you can't do with EK2 in the near future, and the answer is yes.

DR. FREDRICKSON: Dr. Petersdorf?

DR. PETERSDORF: I want to make a philosophical comment. I take great issue with what you said there, Mr. Hutt, simply because if your arguments carry through to their logical conclusion, there would be in fact no biomedical research, because there is no completely safe research experiment. The reason most of us are here and are enjoying the certain benefits of health that we are enjoying is because people have perhaps in their ignorance done dangerous experiments. There isn't anything that you do in biomedical research that doesn't carry some risk.

So I feel that the risks here have to be evaluated or the cost-benefit ratio has to be balanced. I think you have been unduly conditioned by your

previous experience, which I know may have been somewhat traumatic from time to time.

(Laughter.)

Nevertheless, I think that there is a statement that you are making that precludes moving back any of the frontiers of science. To me that is an anathema.

MR. HUTT: I would simply say in response that science can move ahead without, I think, tremendous impediment if there is some degree of control, and it does not preclude placing on those who wish to move ahead the initial burden of showing that there is a reasonable risk, and the lowest possible risk being borne by the public.

DR. FREDRICKSON: Dr. Dodds, you had your hand up.

DR. DODDS: I have listened to the concerns referable to the physical containment, and the concern with the type of host, and I come back to something that to me is very important. The only thing I have heard about the containment of the human being is that if he does something wrong we will slap his fingers and shut off his money.

I am curious--what do you do with the irresponsible investigator, or maybe he doesn't exist. I don't know; I am asking. If he doesn't exist, then the question would be easily answered.

DR. FREDRICKSON: I suppose, Dr. Dodds, not to evade your question, that we are right now trying to define how we would define irresponsibility, and then we can return to your question again in terms of the strength of the guidelines as opposed to regulations and all that sort of thing.

MS. HAYGOOD: I think that E. coli as an organism presents a lot of advantages in terms of safety because it is so well understood, but I think it is also clear that it is not the most desirable type of organism to be used routinely, simply because it is so ubiquitous. I know that this is recognized in the proposed guidelines, and it is suggested that other systems should be developed with the intent that in the long run they should become the standard laboratory organism for as many experiments as possible.

But I don't think that it is clear in the guidelines what sort of incentives there are going to be for the development of these kinds of organisms. I think that everyone is so comfortable with E. coli that if the development of such organisms is delayed, and they arrive on the scene later, we should consider what the scenario is going to be when that happens, and what is the appropriate way of gently moving people away from such a ubiquitous organism.

It is hard to tell precisely how effective the encouragement of the committee in terms of these proposed guidelines is going to be in terms of any real change in the near future in terms of the kinds of experiments that are going on.

DR. FREDRICKSON: Dr. Stetten, do you want to respond to Ms. Haygood's question?

DR. STETTEN: I would like to make one comment in relation to this whole discussion, and that is the one risk which I have not heard described or discussed is the risk of inaction, which I think is a very real risk.

The risks that we are talking about are largely, as has been stressed by others, imaginary scenarios, which may or may not bear relation to reality. The risks of inaction, with a little imagination, can be choreographed equally easily. I think these are risks, not so much costs, but risks which must be taken into the equation before we decide whether to go or not to go.

Some years ago a popular song writer worried about the pollution ambient in this country in a song, the refrain line of which was "Don't drink the water, don't breathe the air." This was good, sound advice, in that if one followed it, one would certainly evade the risks of pollution. Unfortunately, one would also die.

DR. FREDRICKSON: Let me answer your question, then, Ms. Haygood.

The NIH does have certain powers or remedies for the capacity to encourage the movement of research along certain lines, and that if it seemed wisest and best from all considerations, to move gradually toward the development, or rapidly or as fast as possible toward the development of safer organisms and eventually to use only those, this is possible within those powers that we already have. And I guess it is part of the education that we are going through today, to determine how necessary this does seem, and how important.

I see a hand from the public, and before I begin the statements from the public, I will take one unprogrammed statement from the public. Would you identify yourself, please?

DR. GOLDSTEIN: I am Richard Goldstein of the Boston Group.

DR. FREDRICKSON: You are actually to be granted some time a little bit later, Dr. Goldstein.

DR. GOLDSTEIN: Yes, but I thought it was an important point to make. I don't think there is any question here about the freedom of inquiry. It is a matter of freedom of manufacture, of a novel microorganism, and I

think it is a real dangerous precedent to set that seemed to be said before, that oh, someone is going to block this from the course of science. That is not it at all. No one is saying that the research should not go on.

DR. FREDRICKSON: Dr. Hogness, do you want to respond to that, and then we will go on.

DR. HOGNESS: Well, I don't see how you do research without techniques, and what you are essentially saying is that you can determine that without the technique. There are certain things that I have decided you cannot do without that. Therefore you are abandoning it. You are limiting inquiry. I don't think there is any question that there is a tie-in between how you do something and what you do. It is not simply in terms of a manufacture.

DR. GOLDSTEIN: Well, it is a manufacture of whatever kind of system you are going to do it with.

DR. FREDRICKSON: Dr. Goldstein says it is not a question of inquiry, but one of manufacture, and Dr. Hogness, for those of you who didn't hear him, says that manufacture is a technique that leads to inquiry, and there are the horns of that dilemma.

Dr. Rosenblith?

DR. ROSENBLITH: This is not an area in which I have any primary biological competence, but let me just draw on a little experience that I think we have gained in such areas as nuclear technology and the area of space, and the ... accident of the astronauts. That is, in some ways we have felt that as our technological power increases, that there is a need for getting at what seems to be a reasonable commensurability of risk and benefit.

It is very clear that much of what involves the handling of plutonium is both tedious and not very convenient, and yet that is the way it is being done, because there is no other way that we have found so far of doing it. And I do believe that I heard a little bit too much of an all-or-none kind of argument here this afternoon, when that doesn't seem to me the argument.

The argument is what is rational, what sets a reasonable baseline for self-correction as it goes along. I think that if we overinvested in a degree of ignorance that we have today, we would dearly regret it.

On the other hand, if we said today, well, I don't know what micro-organisms be damned, but full steam ahead, the problems that we would face are of a very, very different order. Now, my hope would be that what these guidelines would do is to, one, create a community of education and training such as the one that I think exists in Dr. Petersdorf's laboratory,

and finally, a community that does say and does continuously inquire into the problems of what technological achievement demands in terms of technological assessment. That is really what it seems to me this needs to be about, instead of trying to do something else, which says you prevent me from doing my experiment or you may be risking something.

I don't think that we are looking for a riskless microorganism society, but we are looking for one in which the risk is commensurate with what our understanding is likely to be. I would hope that we might get off this all-or-none kind of a debate.

DR. FREDRICKSON: Thank you for helping us get off that point, because I do want to start another one.

DR. HANDLER: May I add just one sentence?

DR. FREDRICKSON: Yes, Dr. Handler.

DR. HANDLER: The problem, the only one that I can hear is that I don't seem to know what the risk is. By that I mean that if the risks are only those associated with the organisms with which we have been familiar, then we do know how to handle them, and that is the essence of the guidelines. The guidelines have estimated risks on the basis of previous experience with various kinds of organisms and their products, and the question Dr. Sinsheimer, I think, was putting, and others as well, was whether or not the whole is something other than the sum of its parts, and that is, is a form of E. coli into which you have added a plasmid really something with which you have any experience whatever, and whether the manifestations of its infection in plants or animal are those which you have some reason to predict.

The problem is that maybe you can't. I think this is what Bob was saying earlier, and it is the one thing that troubles me. Otherwise I feel very comfortable saying just the kinds of things that Dr. Rosenblith just said. It is just that I am not sure that the prediction of risk and benefit rests on anything known to me.

DR. FREDRICKSON: Very good.

Now, as you know, one purpose of this committee, one of its many purposes was to be sure that interested members of the public would also have an opportunity to speak to this committee. And we have received nine requests to do so. Only one member has indicated a need to leave earlier than most, and it is for that reason that I will call upon him to begin. Otherwise we will attempt to intersperse the presentations more or less in the order in which they were received.

We have written statements from most of the individuals who desire to make them. If they wish to abridge their comments, we will see that their full statements get into the record.

MR. HUTT: Will those be distributed to us?

DR. FREDRICKSON: They will when we get enough of them.

DR. MC CARTHY: We have most of them Xeroxed now.

DR. FREDRICKSON: They are in process.

Now, I just must state the rules for this part of the proceedings, however. At the end of 9 minutes, should you go that far, those of you who are public presenters, I will hold up my hand, and at the end of 10 minutes you really must come to a halt. We will then have approximately 5 minutes for the committee to address questions or comment, for you to have colloquy with them if that is possible.

With that preamble--and we will stop sometime before we hear all nine, I assure you, for coffee--I would like to call upon Dr. David Baltimore, who is from MIT; and while I hope that each of you who come forth will indicate something of the nature of your connection and interest in this particular problem, I will save Dr. Baltimore from reminding any of you who do not know it that he is a 1975 recipient of a Nobel Prize in Physiology and Medicine. Dr. Baltimore, your clock is starting.

DR. BALTIMORE: Dr. Fredrickson, ladies and gentlemen. I was one of the original members of the National Academy of Sciences Committee on Recombinant DNA Molecules, the committee that recommended a voluntary deferral of certain experiments. I was also on the organizing committee of the Asilomar conference, but since that time I have had no role in the formulation of the proposed guidelines, and have really come because I have an interest in these questions and an interest in seeing that this work goes forward under the safest possible circumstances.

In this statement I want to deal with three aspects of the proposed guidelines. The first is whether they are an appropriate response to the hazard posed by recombinant DNA molecules. The second is whether the process that has led to the formulation of these guidelines has been a responsible process, and finally, I wish to emphasize how urgently the international scientific community and the public it serves needs these guidelines.

Let me first deal with the question of whether they are an appropriate response to the hazards in recombinant DNA work. A precise answer to that question would involve a precise knowledge of the hazards involved, and as has been emphasized so often here, the hazards involved in recombinant DNA

work are potential hazards, and no one knows whether any of those hazards might ever exist, even if no precautions were taken. We therefore had to assume where the hazard might lie, and it has been a general assumption that a hazard might be posed if any bacterium or virus carrying a piece of foreign DNA were to become established as a stable part of the flora of the intestinal tract of any human being or animal.

By posing that as a potentially dangerous situation we assume that once a bacterium or virus carrying a recombinant DNA molecule becomes part of the normal flora, it might pose a hazard because the DNA in it might become released or might make products that become released and cause injury to humans, animals or plants.

So when we ask whether the proposed guidelines are appropriate to the hazard, we are really asking if they will guarantee that artificially-joined DNA molecules stay in the laboratory and are not disseminated in the general population.

A second level of analysis has gone into the formulation of these guidelines. In this analysis an attempt has been made to grade the potential hazard of a given recombinant DNA. In line with the recommendations of the Asilomar meeting, the present guidelines attempt to grade a series of protective measures against the potential hazards. The argument is that for innocuous recombinants, the usual laboratory procedures will suffice, because even if those recombinants got out of the laboratory the hazard they pose is no more severe than the hazard posed by the microorganism that contains the recombinant DNA.

I wish to emphasize this point, especially in relation to some of the discussion today. It is not conceivable to me that the mere manufacture of a recombinant DNA poses a hazard per se. The shuffling and mixing of DNA molecules has gone on for eons, and if it were dangerous to add a random piece of DNA to a plasmid or virus, I think we would know that already. I think we must focus on those DNAs that have reason to be thought a potential hazard, and not on the mere joining of DNA molecules.

The proposed guidelines become very restrictive as any potential hazard becomes evident. Taking the hazard that I personally have thought most about, the hazard implicit in joining the DNA from a cancer-causing virus to a prokaryotic vector, let us see what the guidelines propose in the way of safety for the population.

Reading from page 30 of the guidelines, it states, "P4 plus EK2 or P3 plus EK3 should be used to isolate DNA recombinants that include all or part of the genome of an animal virus." What this means is that my proposed

experiment can only be done under one of two conditions. One of these conditions is the type of physical containment used for the manufacture of biological warfare weapons combined with a vector that has an a priori likelihood of one chance in a hundred million of surviving outside the laboratory. It would be possible to lower the level of physical containment only if an organism were used as a vector that had been proven not to get out of the laboratory at one chance in a hundred million. And even then the level of physical containment would be very high.

It seems to me that these proposals offer the public an enormous degree of protection, but at the same time make it possible, and I might say, especially in the context of somebody who works in a university situation training students, only barely possible to go forward. This leads me to a different way of looking at the question of whether these guidelines are an appropriate response to the hazard.

One could argue that the only appropriate response to this unknown potential hazard is to completely ban the experiments. After all, that would provide the highest degree of protection of all, so there must be an argument why these experiments should be done at all, and I think that argument needs to be remembered in very forceful terms.

There are two critical reasons why these experiments should be allowed to go forward with the appropriate protection. One reason is because of the scientific interest in the experiments, and the second reason is because of the medical potential in them. The ability to form recombinant DNA molecules opens up the opportunity to utilize the enormous resources developed in the study of bacteria to probe how higher cells function. The medical justification is twofold. There is a potential for the manufacture of biologicals, and there is a potential for understanding the complicated diseases that arise from the malfunction of cells.

Such cellular diseases are the major unsolved medical problems of the developed world. Foremost among them is cancer, and I think it is important to remember one thing about cancer, because I have had an argument made to me that since cancer is an environmental problem one needn't worry about attacking it by sophisticated biology; one can attack it by getting rid of problems in the environment. However, the statement that 80 percent or more of human cancer is caused by environmental influences, while true, does not mean that 80 percent of the disease is caused by things in the air we breathe or the water we drink. It rather means that 80 percent of the disease is caused by various aspects of our lifestyles, including our diet, our smoking habits, our sexual habits, et cetera. Human beings are very conservative about their personal habits and do not easily change them. Even if we identify the causes of breast cancer, cervical cancer, prostate cancer, colon cancer, and bladder cancer, it is unlikely that we are going to be able to design a civilization that will be acceptable to the population and that will prevent the occurrence of these terrible diseases. We

should certainly make every effort we can to understand how our lifestyle causes cancer, but we must also push forward on a more basic attack on the problem, and I believe that attack is made much easier by the ability to manipulate recombinant DNA molecules.

Let me turn to another aspect of the guidelines. Has the process by which the guidelines have been arrived at a responsible one? Dr. Berg has outlined the history of the process that brings us here today, but we should all realize that the process is a unique one. It represents an attempt by a large segment of the community of biologists to play the role of public advocate. When we talk about public involvement in the decision-making process about science, I could not agree more that policy questions should not be left to scientists, but I think it is important to realize that when one is talking about a technical issue it is extremely critical to have public advocates among the specialists.

The process outlined by Dr. Berg is a process by which a large proportion of the community of molecular biologists made themselves into public advocates and tried their best to protect the citizenry against the kinds of dangers that might potentially arise from their own research work. I think the final step, the hopefully final step in the process of generating these guidelines -- that is, the meeting we are at today -- is part of a very necessary process by which the public asks whether the advocates have in fact been advocating the needs of the public.

Ultimately the public must decide what it wants and the scientists must translate those wants into specific policy.

There is another aspect to the question of whether these guidelines are a responsible reaction to the potential threat posed by recombinant DNA work. Is it a sufficient response to release the guidelines for study sections to follow? Will that action guarantee that all recombinant work goes forward in a safe manner? There I feel the answer is no. All granting agencies, such as the American Cancer Society and the National Science Foundation, must adhere to the guidelines. And very importantly, industrial laboratories that have an interest in recombinant DNA work should publicly announce their intention to work within the guidelines.

I think that anyone reading about a recombinant DNA experiment in a primary scientific journal should expect to find a statement about the safety aspects of the work. Journal editors should insist on such statements. Such a process would be, I think, the best safeguard against experiments that do not follow the guidelines because of the strong peer pressure on anyone reporting an experiment that was done without the appropriate containment.

Dr. Fredrickson, here I think it is part of your responsibility in this matter to see that the guidelines are widely followed by establishing the appropriate procedures.

Finally, I wish to emphasize the need for haste. The Gordon conference is now almost 3 years ago, but we are still in the process of generating a set of guidelines under which the work can be done. During this time many experiments have been done, but many critical experiments have been avoided and many laboratories around the world have been marking time.

In summary, I think recombinant DNA research is critical to the progress of science, and to the solution of medical problems. I think the present guidelines provide a very conservative framework within which such experimentation can go forward. I think these guidelines have been arrived at by a responsible process in which scientists have been acting as public advocates. I feel that the world has waited long enough for the issuance of these guidelines, and that with as little delay as possible they should become national policy.

Finally, I hope that other agencies that fund biomedical research will adopt these guidelines, that industrial laboratories will publicly announce their adherence to the guidelines, and that individual scientists will accept them as a framework within which all recombinant DNA experiments will be formulated.

Thank you.

DR. FREDRICKSON: Thank you, Dr. Baltimore.

Are there questions or comments for Dr. Baltimore.

Dr. Sinsheimer?

DR. SINSHEIMER: I just wanted to ask about one point. You made the argument that you felt that we should focus only on DNAs that have some reason to be thought of as hazardous based on the position that the random combinations of DNA must have occurred, and we are still here. I agree that it is probable that random combinations of DNA may have occurred at some sporadic frequency, some frequency we do not know, and the consequences of such events we may not know.

Unfortunately that doesn't give me a great deal of comfort because it sort of reminds me of saying well, the human species evolved in the presence of background radiation, and therefore we don't have to be worried about radiation, whereas what is being contemplated, at least, is an increase in those events certainly by many orders of magnitude.

DR. BALTIMORE: The argument I draw partly from an argument that was made much more broadly by a guy from Oxford, whose name I have forgotten. He said he felt that all DNA had been joined at some time -- Walter Bodmer -- that all DNA had been joined at some time in history, and you didn't have to worry about anything. I certainly wouldn't go that far, but I would say that for instance, the joining of random bits of the E. coli chromosome back to plasmids has certainly gone on. The joining of the DNAs that form a family of genetic transfer have certainly gone on, probably with some reasonable frequency, because you can isolate them in the laboratory if you try.

The kind of random DNA effect, by inserting random DNAs into plasmids, the ability to cause a high level of pathogenicity that way strikes me as extremely unlikely. One has to worry about more focused problems than those.

I would never say that anything is 100 percent impossible, but when I am looking at where does the danger seem to me and what dangers should we be responding to in a defined way, I don't think that the mere insertion of DNA is that dangerous.

DR. SINSHEIMER: We don't know what the historic frequency of such events is, and not all things in the world are linear.

DR. FREDRICKSON: Any other question or comment for Dr. Baltimore?

MR. LADWIG: Just a point of clarification. Were you saying that you think the scientists as a public advocate was sufficient to keep tabs on this type of thing and that public input from outside science was not needed?

DR. BALTIMORE: By no means.

MR. LUDWIG: I don't think I heard that right.

DR. BALTIMORE: I explicitly said that I felt that input from outside of science is needed. What I am trying to do is to put in a somewhat different context the events over the last few years, because I am effectively responding to a charge that hadn't been made here, but has been made widely elsewhere, that by having only scientists involved in the process, the public's needs are not being served. And I don't really think that is true. I think that is making us into the kind of people whom we think exist in large industries, for instance--I am trying to be as gentle as possible--

(Laughter.)

DR. HANDLER: Your guidelines are a lot more likely to be observed meticulously in large industry than in the university.

DR. BALTIMORE: Oh, I completely agree with that.

DR. HANDLER: Be careful about how you denigrate industry.

DR. BALTIMORE: I said I was trying to be gentle, but I don't think we are looking at an adversary situation in which it is the scientists against the public or the scientists trying to grab for something for themselves. I really think--in fact, I am absolutely sure--that especially in the formulation of these guidelines, you have seen scientists at work trying to do what they felt was right for the public, not right for themselves. Right for themselves was very clear. It would have been to shut up as soon as you realized there was a problem if all you wanted to do was get on with your work.

What you have seen here is an historic process by which everybody in the community has not shut up but, in fact, has been mulling it over in their minds, talking about it, working it out, meeting in committees, taking a very large amount of their time.

DR. FREDRICKSON: Thank you, Dr. Baltimore.

I am going to call now on Dr. Charles Madansky from Harvard, and following Dr. Madansky's statement and discussion, we will then have a short coffee break.

Dr. Madansky, would you tell us your affiliation at Harvard?

MR. MADANSKY: First thing, I am not a doctor yet. I am a graduate student.

DR. FREDRICKSON: That's all right, we are all in this together.

(Laughter.)

MR. MADANSKY: I am doing research on the genetics of an animal virus at the University of Massachusetts Medical School. I am actually a graduate student in the Department of Microbiology and Molecular Genetics at Harvard Medical School.

And I am here because I am sincerely concerned over the potential hazards involved in the use of DNA recombinant technology as regulated in the present set of proposed guidelines, and what I say now represents as well the opinions of many concerned scientists, including the Boston Area Recombinant DNA Group, and the Genetics and Social Policy Group of Science for the People.

The long and difficult job of drafting these guidelines was a truly admirable endeavor. As someone who comes in daily contact with people doing this type of research, I feel very strongly that now is the best time to improve and tighten these guidelines in spite of the growing impatience of industry and certain researchers to get on with the work.

I believe we should delay this work until the present guidelines are altered in at least three basic ways. One, we should not use E. coli, whose natural host is man, as the vehicle for cloning. Two, the low classification for shotgun experiments in invertebrates, some pathogenic bacteria, and plants because of potential biohazards should be altered. And three, we should create effective, board-based agencies for the implementation and monitoring of safety procedures recommended in the guidelines.

Now E. coli is a normal constituent of the human gut and pharynx. Any potentially dangerous organism which this technology generates is capable of infecting the entire human population. The guidelines provide standards which theoretically lower the probability of danger through the use of crippled E. coli and physical containment, but these probabilities are not low at all considering the number of bacteria in any one experiment, the number of experiments done over the years, the possibilities of uncrippling taking place by genetic transfer or reversion, and the known weaknesses of using P3 or P4 containment. Therefore, it is unwise to use an organism whose ecological niche is human beings, like E. coli.

Many shotgun experiments or blindly cloning total DNA from invertebrates like insects or pathogenic bacteria carry clear hazards. Here P1 or P2 containment which represents little more than standard microbiological technique, and Ekl, or normal E. coli, are the standards for safety. Yet insects carry many known DNA viruses, some similar to those causing tumors in vertebrates. Invertebrates also code for hormones with potential activity in human systems, for instance, nerve growth factor, as well as enzymes whose continued synthesis in the human gut would be disastrous, like cellulose-degrading enzymes, thus causing a victim to have no roughage.

For each of these known potential hazards, there must be others among the many as yet undiscovered genes in these organisms. The fact that the experiments with these organisms are at present being carried out is frightening to me.

As a graduate student I know that we are often socialized into accepting the poor safety habits of the lab where we work. We can't depend on the people who are doing these experiments to check on their own safety conditions. They have already ignored or accepted the risks involved. But those people most likely to be exposed to a risk--technicians, janitors, graduate students, and dishwashers--should have a right to protection under the Occupational Safety and Health Act.

Therefore, we recommend that independent safety and inspection committees be set up with a broad-based composition representing the interest of all people concerned.

To me there are at present no pressing social benefits to be gained from this research. If they are to come at all, what matter if it takes 20

or 25, 50 or 55, 100 or 105 years? I implore you to take the time now to make the significant changes in the standards for biological experiments which will spare us needless regret and human misery in the future.

DR. FREDRICKSON: Thank you, Mr. Madansky.

Mr. Hutt has a question.

MR. HUTT: I just have one technical question. There was a list in the submission that you or the so-called Boston Group made of things that should be prohibited. There is also a similar list in the guidelines. I am not technically competent to determine whether those two lists are the same or different, and if differences exist, would you explain why your list is longer? All I could do is add the numbers, and you seem to want to prohibit eight things and the advisory committee guidelines prohibited six.

Dr. Singer, I might ask you the same question. I simply could not correlate them. Can you describe the differences between your two lists or are they the same?

MR. MADANSKY: Right now I don't have that list in front of me, but I can say that there are some clear differences between the two lists, and those experiments which in fact have gone forward in various laboratories in California and Boston with invertebrates--that is, insects like Drosophila melanogaster--which we are asking that they be considered vis-a-vis their potential hazard by not going ahead under what we consider to be too low classifications. There are many differences, but I can't go into them all.

DR. TALBOT: There are four items essentially the same on the two lists--that is, prohibited by both. Two of the items that are prohibited in the La Jolla guidelines aren't on the list from the Boston Area Recombinant DNA Group. Four of the items on the latter list are not prohibited in the La Jolla guidelines, but they are discussed, and containment guidelines are specified.

MR. HUTT: Could we at some point, and this may be an inappropriate point--you may want to defer this, Don--but perhaps Dr. Singer could explain why the four on the Boston list were not on the NIH guidelines at some appropriate point, whenever you would like.

DR. FREDRICKSON: I think it is appropriate now to take this up, because such analysis is available. Dr. Singer?

DR. SINGER: I could certainly do this, but I would like a few minutes to sort out, and I would like to be sure I am looking at the right lists before I start. That is page 3?

MR. HUTT: Page 3 of theirs and page 11 of yours.

DR. SINGER: Okay. If I could spend a few minutes looking at this, I would be glad to respond to your question.

DR. FREDRICKSON: Now, theirs refers to a statement from the Boston DNA Recombinant Group?

Dr. HUTT: Yes.

DR. FREDRICKSON: Which document we offered earlier here for distribution.

Why don't we give Dr. Singer the coffee break?

(Laughter.)

We will promptly reconvene at 3:45.

(Brief recess.)

DR. FREDRICKSON: Dr. Allan Silverstone of MIT had prepared in his remarks a partial analysis, at least, of the differences between the La Jolla guidelines, that is, the last version, and the statement from the Boston Area Recombinant DNA Group. The question which actually was asked at the time we adjourned. So we are granting him a portion of his time to give his perception of the differences between the committee's guidelines and this submission from the Boston Group, and then we will return to Dr. Singer's analysis of what differences remain.

Dr. Silverstone? Would you go to the podium, please?

DR. SILVERSTONE: I have more extensive remarks on other matters, but I thought maybe since part of my remarks included comments on the differences in containment, I might make them, and then Dr. Singer could reply and elaborate.

The major difference between the Boston Area Recombinant DNA guidelines and those proposed in La Jolla is that we propose the banning at this time of additional experiments, shotgun experiments, in mammals and avian species. We also propose much higher levels of P4 containment for shotgunning of the other organisms down to prokaryotes.

We similarly propose that physically purified, 99 percent purified DNA from mammals and avian species also be banned.

Our reasoning is that we believe the original idea of banning some of these experiments was that there might be DNAs that could cause cancer in

humans or other organisms. There might be DNA that would produce--whose gene products would be hormones that could act on humans or other organisms, and also that the DNA derived in a shotgun experiment could produce enzymes that would create various kinds of biological havoc. I think the remarks of Dr. Curtiss about the fact that we should consider much more than just humans as targets, but the entire biosphere, is probably reasonable.

I think the main point is that a shotgun experiment does just that, it is a shot in the dark. You don't know what you are picking up, and until you have grown out the cloned DNA, you don't purify it initially, you have to grow up a fair amount of DNA before you know what you have. This would be on the order of 10^8 to 10^{10} such genetic units.

We therefore feel that these kinds of experiments should be treated with the utmost caution and in the main should be banned.

DR. FREDRICKSON: You are referring then to the document entitled "Recombinant DNA Molecule Advisory Committee Proposal," in which you discuss differences between the Asilomar statement and Woods Hole guidelines.

DR. SILVERSTONE: Those were proposed based on the Woods Hole guidelines for the La Jolla meeting. [See Appendix A to these Proceedings]

DR. FREDRICKSON: Yes.

DR. SILVERSTONE: Now, in the La Jolla meeting, as people can see from the document, different proposals were made and voted on. Our position continues to be that certain experiments should be banned, that those guidelines, the La Jolla guidelines, instead set standards for containment, but gave the go-ahead to do. [See Appendix A to these Proceedings]

DR. FREDRICKSON: Very good, I just wanted to clarify your point.

Dr. Singer, are you ready then to discuss your perception of how the La Jolla guidelines, that is, the last edition, compare with the requests of the Recombinant DNA Group from Boston?

DR. SINGER: I might just say that this document which Dr. Silverstone referred to was available during the La Jolla meeting, and I think perhaps the first thing we could do would be to compare the list in the document, which is on page 3 of the document, which is entitled--I guess this particular one is not titled, but it has a covering letter signed by Duncan, Goldstein, Orrego and Primakoff. It is dated November 24.

DR. FREDRICKSON: I want everyone on the committee to realize where we are: page 3 of that document, yes.

DR. SINGER: Okay, so perhaps the first thing to do would be to look at those items in this list of experiments not to be performed, which in a sense are also listed as experiments not to be performed in the proposed guidelines as they came out of La Jolla. Then we will know what remains as a matter of differences.

DR. FREDRICKSON: This document is not in your brown book. Some of you do not have it.

DR. SINGER: The cover sheet on the whole package is dated the first of February 1976, and it is a memorandum from the Department of Microbiology and Molecular Genetics, Harvard Medical School, addressed to the members of the advisory committee. I believe it was mailed to everybody.

Well, in any case, I will try and go over it. Could someone turn on the slide projector, please, and put back in the slide (2, page 38) that shows the list of experiments as they are in the La Jolla document that are not to be initiated at present?

Slide 2

Experiments Not to be Initiated at Present

1. Experiments involving DNA from classes 3, 4, and 5 (CDC)
2. Deliberate formation of recombinants when genes for dangerous toxins are present.
3. Deliberate formation of recombinants from plant pathogens if likely to increase virulence or host range.
4. Widespread release into environment of any organism containing recombinant DNA, except when no reasonable doubt of safety.
5. Transfer of drug resistance traits to new microorganisms, if transfer could compromise drug use in medicine or agriculture
6. Large scale experiments if harmful products made. Exceptions possible with permission of Advisory Committee.

The first one, which are experiments involving DNA from classes 3, 4, and 5 of the CDC classification is also listed in the document to which I am referring on page 3, so that is one in which the two agree.

The second one, the deliberate formation of recombinants when genes for dangerous toxins are present, is also listed in this list, but it is actually listed, as I understand it, as two things, because toxins of bacterial or animal origin appear to be separated from toxins of plant origin, whereas the La Jolla guidelines put the two together just with dangerous toxins. So that says that number 6 and number 8 on this page 3 are essentially taken care of here.

Number 7 on page 3, which is the formation of recombinant DNA molecules where part of the recombinant is from plant pathogens whose virulence or host range might likely be increased is included under number 3 of the list that is on the slide.

Okay, now, number 2 in the list on page 3 is the introduction of DNA coding for resistance to antibiotics into any bacteria in which this drug resistance does not naturally occur. That is similar to number 5 on the list that is on the slide, except that number 5 is qualified for those drugs of importance in medicine and agriculture, but basically, in my view, I would say that they are very similar.

MR. HUTT: Well, is it the same or is it just similar?

DR. SINGER: Well, they are not the same, no. The one in the La Jolla guidelines is qualified, but on the other hand it is true that there are a large number of antibiotics which are antibiotics by definition but which are not useful for various reasons. So I am not sure that substantively that is an important distinction.

MR. HUTT: Theirs is broader than yours?

DR. SINGER: That is right. It would presumably include antibiotics which are also not useful for various reasons.

Okay, now -- so now to focus on things which are on this list on page 3, which are not included in the La Jolla document as summarized on this slide, first of all, number 1 on page 3 reads, "The introduction of the DNA from mammals or any warm-blooded animals into E. coli. [This applies to total DNA and 'purified' segments.] The possibilities of introducing tumor virus genomes, enzymes converting bacterial metabolites to small molecule hormones and many others make such experiments extremely hazardous."

Okay, those experiments are not listed as experiments to be deferred at the present time. This is the slide which I showed you before (9, page 47) which summarizes the position that the guidelines take on this particular aspect--namely, for mammals and warm-blooded animals as a source of foreign DNA and introduction into *E. coli*. This is divided up rather than all being together. The mammals are divided into primates and other mammals, and then the other warm-blooded animals are the birds, so that we are concerned with the comparison between the request on page 3 that such experiments not be done and these particular guidelines.

So for the primates, as I pointed out before, the guidelines recommend P3 physical containment with an EK3 host-vector system, or P4 physical containment and EK2 host-vector systems, except in the case of where the sources of foreign DNA are embryonic or germ-line DNA for the reasons I outlined before. The advisory committee concluded that such DNA presented less of a hazard primarily because the possibility of introducing DNA from viruses or other adventitious agents is lowered.

Other mammals are at P3 and EK2; and birds, which are the remaining classification of warm-blooded animals, are also at P3 and EK2. This has been summarized for you where the guidelines are as opposed to the point made in this document that such experiments be banned.

Slide 9

Guidelines for Experiments with *E. coli*

"Host-Vector" Systems. I

'Foreign' DNA = Mixed Fragments = "Shotgun"

Foreign DNA Source		Containment	
		Physical	Biological
Eukaryotes:	Primates	P3	EK3
		P4	or EK2
	Embryonic	P3	EK2
	Other Mammals	P3	EK2
	Birds	P3	EK2
	Cold Blooded Vertebrates	P2	EK2
	embryonic	P2	EK1
	Lower Eukaryotes	P2	EK1
	if pathogenic	P3	EK2
	Higher Plants	P2	EK2
	if pathogenic	P3	EK2

Okay, now on page 3, item number 3, it says the use of currently available animal viruses as vectors are also not to be performed. I didn't put this slide back in the machine, but you will recall that I summarized it this morning. We could have the lights now, I think, please.

Two animal virus vectors are discussed in the guidelines, polyoma and simian virus 40, and distinction is made between the containment afforded by the two of them. Polyoma is not known to infect humans or human cells in tissue culture, therefore the conclusion was made that it was unlikely that polyoma virus particles containing recombined DNA fragments would escape from laboratories in a meaningful manner, because of the inability to infect humans, and therefore polyoma could be used as a vector under the specified conditions, which were P3 for any non-pathogenic organism, and P4 for any pathogenic organism in class 2 of CDC, with P3 being allowed if no virus particles were being made in the experiment, and with the additional proviso that a defective polyoma had to be used as the vector, and not an intact wild-type polyoma.

Similar considerations are for SV40, but with the added proviso that because it infects humans and therefore can be carried out of laboratories, the physical containment requirements for experiments with DNA derived from non-pathogenic species were at P4, and there were no other experiments permitted except experiments where no virus particles were made, which could be done at P3, or where the DNA was highly purified and shown not to contain harmful genes.

So this then summarizes for you the position the guidelines take on this same point, which is number 3 here.

Okay, another point of disagreement is item number 4 on page 3--the introduction into E. coli of the whole genome or any purified segment of a viral DNA which is oncogenic in any species. Also, the formation of hybrids of total or partial oncogenic virus DNA molecules, with E. coli vectors, whether or not these hybrid molecules are subsequently intentionally cloned in E. coli.

The guidelines make no distinction between oncogenic viruses, oncogenic animal viruses, and any other animal virus in terms of the introduction of such--of DNA derived from animal viruses into E. coli. All such animal viruses are treated together.

If anybody wants to look at this in the guidelines, it is under c(1). I haven't got the page, maybe somebody could find it.

SPEAKER: Page 11.

DR. SINGER: I have the summary. It is on page 6 of of the summary. Okay, it says there that if the foreign DNA is from any virus that infects

animals that P4 physical containment and EK2 are required, or P3 and EK3. However, after purification by cloning and the demonstration that only a segment of the viral DNA that contains harmless genes is involved, then containment can be moved to P3 and EK2. So that the experiments are permitted, but under what could probably be characterized as the strictest containment requirements for any experiments.

The only other item on page 3, as I understand it, that is different is number 9, which says that any recombinant DNA molecule or strains carrying them mentioned in items 1 through 8 which have already been made should no longer be used, but rather be autoclaved, et cetera.

The guidelines as such do not refer to the disposition of anything that has been made thus far but which would be interdicted under the experiments listed as not to be done in the La Jolla guidelines.

Did I muddle that, Don? You look slightly confused.

DR. FREDRICKSON: Slightly.

DR. SINGER: Okay, I will say it again, then. The La Jolla guidelines do not contain a statement comparable to number 9 relative to those experiments which are on the list of experiments not to be done in the guidelines. However, the advisory committee discussed that problem at the meeting at La Jolla, and, as I recall, the decision that they made was that any investigator who has such materials needs to bring them to the attention of the advisory committee, and the advisory committee, together with the investigator would presumably decide on the proper course of action.

MR. HUTT: Why wasn't that statement that you just made in the guidelines?

DR. SINGER: I am not sure whether it was considered a procedural matter, as was, for example, the certification of EK2 host-vectors, so that it is not a formal part of the guidelines, but in a sense it is not really different because, as I understand it, the guidelines are basically the minutes of the meeting, and that statement occurs in another part of the minutes.

MR. HUTT: It occurs in the minutes?

DR. SINGER: It is in the minutes of the La Jolla meeting.

MR. HUTT: They have no effect.

DR. FREDRICKSON: I am sorry, Peter?

MR. HUTT: The minutes have no effect on the guidelines, obviously.

DR. FREDRICKSON: Dr. Goldstein, and then Dr. Hogness.

DR. GOLDSTEIN: Just two brief things, one especially on that point number 9.

DR. FREDRICKSON: Please move toward the microphone.

DR. GOLDSTEIN: I think number 9 is a very important point, and we just talked about what happens to these strains of vectors which have been made already. There are innumerable labs with these. We have now gone through 1 page of a 20-page critique that we wrote. There are many other differences, and I don't know if we have time now. I think Maxine did a very good job of going through that.

[See Appendix A to these Proceedings]

DR. SINGER: I am sorry, I understood that page was the target of the question.

DR. FREDRICKSON: I think that was the only target you could have hit.

DR. GOLDSTEIN: Sure, absolutely, but I just wanted also to make that point.

DR. FREDRICKSON: Well, actually, if I read the documents carefully, the two major ones, or the two things not yet dealt with, were the request from your group that work with E. coli be gradually phased out, clearly by the end of some period of time, 2 years or so.

DR. GOLDSTEIN: At least.

DR. FREDRICKSON: And then you had some issues about quality control that are further on in your document.

DR. GOLDSTEIN: Well, if you turn to page. . . . I think it is really the second page of the central points in our proposal, which comes right after the cover letter, in the summary of the whole thing, and it deals with such things as we really feel there is no difference between P1 and P2. I even question P3 as a real containment facility, but I think it is important at least to have some kind of psychological effect on the people working in a P3 lab. There are lots of other points like that.

DR. FREDRICKSON: I think it would be better if we prepared that analysis and it were presented in a much more compact way.

Dr. Hogness?

DR. HOGNESS: In response to Mr. Hutt's point, I think the committee's idea was that we were drafting guidelines to guide future research. The

members of the committee could in no way be totally aware of all the recombinant DNAs that had ever been made, some of which might be ones that should be autoclaved and others which pose no hazard. Therefore, as I guess is within the charge of the committee, a motion was made that anybody wishing to continue to use recombinants that were constructed with conditions or host-vectors with less than what the guidelines now specify ought to come before the committee and allow the committee to evaluate the situation, in which case the committee could say autoclave it all, or it appears from the information you know that you can continue to use those recombinant clones. This is why it was not written into the guidelines.

MR. HUTT: Well, there is nothing to preclude that being a condition of future funding of research by that laboratory.

DR. HOGNESS: Yes, right.

DR. FREDRICKSON: Is there any further comment on this matter?

Well, I think, Dr. Goldstein, if you had the time this evening, you will be us tomorrow, and you care to go through the present La Jolla guidelines, and indicate those areas where they still seem at variance with, those important areas, with what you have written here, then you would be performing a great service for all of us, so that we might clearly have on the record and understand where those differences lie, and we could take that up in the morning.

DR. BERG: Let me ask that you include in that charge better description of what the word "banned" means. Forever? And if so, is that to include the fact that even if as we anticipate safe hosts and vectors will be on hand, is the work still to be banned forever? Because I think there is a question of what assurance we have that any host or vector system would ever come anywhere near close to matching what we already can produce or conceivably would produce without E. coli. So the word "banned" I find very difficult to understand. How does the timeframe work? and also what the alternative is.

DR. FREDRICKSON: Dr. Goldstein had previously requested to speak, and had agreed to wait until tomorrow, and we will, if we may, devote much of our presentation tomorrow or as much as we can to these questions.

DR. GOLDSTEIN: I will deal with the guidelines.

DR. FREDRICKSON: Very good.

DR. GOLDSTEIN: I still would like to sort of - I had a couple of other issues that I wanted to speak about.

DR. FREDRICKSON: We will see that you have time for those as well. But we do need to have an airing of this issue and I think that you should attend, please, to the comments of Dr. Berg about this.

Yes?

DR. BROWN: My name is Donald Brown. Do I understand from your comments that you are going to come up with another variorum edition? There are probably other people out here who have different suggested guidelines. Is it your idea that you wish to give equal time to all of these?

DR. FREDRICKSON: Well, the reason that I have taken the trouble to do this is that we have now as a matter of record this submission from the group, Dr. Goldstein's group. It has been presented to us; it naturally raises issues of variance.

We probably will not have time to open another variorum, but if in your presentation and others that occur--

DR. BROWN: I have a Xerox copy over there.

DR. FREDRICKSON: All right. Indeed, I think that we are moving now toward these major differences between what we have presented and other perceptions of how they might be different. We can perhaps in the course of our discussion now, particularly tomorrow, leave some time for discussing some of those points. But we will not go through a whole variorum edition, and cannot. Therefore, I hope we restrict ourselves to what seems to be the important issues. This is, again, a matter of conception, but I would ask that you try to do so.

Now, I would like to call upon Dr. Susan Wright from the University of Michigan.

DR. WRIGHT: As a historian of science, I have been interested today by much of the discussion--

DR. FREDRICKSON: We can't hear you. Please do speak up much more.

DR. WRIGHT: As a historian of science, I have been interested today by how much of the discussion, especially this morning, has focused on two aspects of recombinant DNA research--first, the molecular aspects, in which DNA is treated primarily as a combination of inert particles, and secondly the possibility of biological containment by manipulation of the genes of E. coli.

Both approaches, it seems to me, ignore something fundamental, namely the fantastic capacity of living organisms to adapt and to survive. This seems to me to be a serious concern in that we may lose sight of the fact

that what we are considering today is not simply another research tool, but a radical change in our ability to manipulate our environment and, indeed, ourselves, too.

Recombinant techniques have been compared to the release of energy from the atom, both in terms of power and in terms of the destructive effects on the environment.

There are two broad categories of questions which may be asked with respect to recombinant DNA techniques, or for that matter with respect to any other form of research which entails hazards to human life.

First, what is the nature of the risk? Second, is the risk acceptable? The questions are very different in nature. The first is a scientific and technical question. As such it must be resolved under appropriate circumstances by members of disciplines which can make a contribution to resolving it.

The second question is quite different. It is ethical in character. As such it is a question which no single group is inherently more qualified than others to ask and to answer. It is unfortunate, I think, that in discussions of the implications of recombinant DNA so far, as well as in the decision-making process, that these questions have been confused, but I think this was also reflected in the discussions particularly this morning.

Given the radically different nature of these questions, and the different qualifications which are appropriate for answering them, two separate but related decision-making processes are desirable.

Up to this point, as we know, the principal decision-making mode, de facto if not de jure, has been the drafting of guidelines by the NIH advisory committee. I shall discuss the contribution of this committee to the decision-making process from three perspectives.

First, in terms of the operation of the committee; second, the composition of the committee; and third, the theoretical basis of the guidelines.

With respect to the first question, it seems to me that in terms of the operation of the committee the questions with respect to the nature of risk and the acceptability of the risk have been confused, for in being charged with the task of formulating guidelines the NIH committee was operating under the assumption that the research would go on, no matter what. In other words, the issue of acceptability was pre-judged.

That claim could be disputed. It could be claimed that the NIH committee would, if it thought it necessary, advise that all forms of research be discontinued. My response to that claim brings me to the second question, the question of the composition of the committee.

An examination of the composition shows that the means for decision with respect to the guidelines have been concentrated in the hands of those who have an interest in going ahead with the experiments. Approximately half of the members of the committee are currently actively engaged in the aspects of recombinant research. Several others have an interest in using recombinant techniques in the future. It would seem unlikely to me that a committee with this composition could judge the hazards unacceptable. By saying that, I am not questioning the integrity of the members of the committee, but it is well known that scientists often have a greater tolerance of hazards associated with their experiments than the members of the population at large.

It is advisable then, in principle, to eliminate the possibility of conflict of interest by restricting the number of those who are engaged or expect to be engaged in the experiments under review.

There is a second aspect of the composition of the committee which is also questionable, and that is the range of scientific perspectives represented. With a few exceptions, the members have backgrounds in genetics, in molecular biology, but where one works within the confines of a scientific discipline it is difficult to understand, and sometimes even to notice the significance of phenomena that fall outside the range of assumptions one is working with. In science this is normal, where when one deals with something very complex one can only focus on a limited part of it at any time. Other phenomena must be ignored.

But in defining the nature of a risk, that is precisely what one cannot afford to do. This is why it is essential that the membership of a committee charged with judging the hazard be drawn from all the perspectives which bear on the problem. In this case, that should include ecology, epidemiology (there is one epidemiologist), biosafety, occupational safety, public health, certainly probability theory, and a discipline which transcends scientific disciplines such as philosophy or history of science. There is some vested interest there, perhaps.

Ideally then, the composition of an advisory committee to judge the risks would be very different from the present composition. It would be far more diverse with representation in the fields mentioned, and in addition the number of members presently involved in the experiments would be limited to a small minority, two or at the most three in fifteen, say.

The third set of questions I want to address concerns scientific basis of your guidelines. There are specific problems such as the efficacy of physical and biological containment and the question of whether the so-called low and moderate risk experiments are actually much higher risks, which critics of the La Jolla guidelines pointed to.

I shall focus here only on the general criticisms which may be made of the scientific basis of biological containment.

The most obvious difficulty is that the experiments of Dr. Roy Curtiss have yet to be accomplished, consequently the scientific community has not had a chance to provide a critique of his experiments and to run independent checks. In saying this I am not at all questioning Dr. Curtiss' expertise or qualifications, but the unpublished experiments of the single scientist do not, in my opinion, constitute a body of knowledge shared by the scientific community which it is desirable to have as a foundation for guidelines.

In view of the serious nature of the risks entailed by recombinant DNA research, I would therefore recommend the following. First, a clear distinction must be drawn between the problem of assessing the risk and the dangers of the research and the problems deciding whether the risks are acceptable. These two issues should be considered by a much wider range of people than hitherto, including those who have raised serious and responsible questions about the research.

It is important to avoid a one-sided predetermined decision.

Second, it seems that a complete overhaul of the decision-making process at the national level is urgently needed. And before a final decision is reached on recombinant DNA, I would suggest that two committees to consider the separate questions of the nature and acceptability of the risks be set up prior to review and revision of the guidelines which have been drafted up to this point.

The first committee should have a far more diverse membership than that of the present NIH committee along the lines suggested earlier. That committee would deal with the scientific and technical aspects.

A second committee on the acceptability of the risks would have a very different composition, reflecting the ethical character of the question of acceptability. This membership should be drawn from a broad range of fields, including the humanities, philosophy, ethics, policy areas such as law and administration, medicine and science. To provide a safeguard against the problem of conflict of interest an essential restriction would be that no member of that committee could be engaged in or have the intention of being engaged in the experiments under consideration.

In general, I would advocate a slower and more cautious approach to recombinant DNA experimentation, and the maintenance of the moratorium until more is known about the risks. While in my opinion many of the claimed benefits are dubious, the risks seem relatively clear.

It has also been pointed out by scientists in the field that there are other ways, perhaps slower, of gaining the understanding of genetic mechanisms which is expected from recombinant techniques.

Finally, there is the question of how members of the committee present today will act. Do they accept the strong presumption underlying most of the presentations this morning that the risks are acceptable and the research will go on, or will they question that presumption and do what in my view they should do, namely to ask whether it is justified at the present time to press forward and to foster proliferation of laboratories and experiments of unknown risks before a more viable decision-making process has been set up, and before the scientific community has had a chance to judge and question the soundness of the scientific foundations of the guidelines.

Thank you.

DR. FREDRICKSON: Thank you, Dr. Wright. Would you like to remain there at the podium for a moment? Perhaps there are some members of the committee who would like to ask a question.

I see two-- You aren't really the public, because you were asked to be here, Dr. Berg.

DR. BERG: I would like to point out one thing that I thought was obvious in Dr. Singer's presentation. A very large number of experiments which require P3 and EK2 are in effect not doable at the present time.

Now, you made some comments about how an EK2 vector will in fact become available to be certified. Dr. Curtiss has told us about his progress. The guidelines say that no experiments can be done that require an EK2 vector unless, until the vector has been certified by the committee. So at the present time it is not dependent on Dr. Curtiss' word--that, in fact, that organism would be distributed widely to the scientific community and in fact will be verified as to whether it satisfies the criteria; and only after the committee, the NIH committee certifies it as such, which is in fact the equivalent of having published it, having it verified independently by many laboratories, will in fact experiments that require EK2 be possible.

So I think that I would like to correct what I think is a misconception, that Roy Curtiss' word up here, saying I may have made an EK2 vector, frees everybody to go ahead and do the experiments without any independent verification.

DR. WRIGHT: Could I respond to that?

DR. FREDRICKSON: Yes, of course.

DR. WRIGHT: With all due respect, Dr. Berg, I think there is a difference between publishing the E. coli and publishing the results of the experiments.

DR. BERG: They both will be. The material and documentation will be published and the organism will be made available to others to verify his results.

DR. WRIGHT: Well, presumably there is a great difference between running tests on E. coli and using it in experiments. As I understand what you are saying, you are claiming that the E. coli should be used in the experiments as a means of running a check?

DR. BERG: No.

DR. CURTISS: I am not sure--I mean, I agree with what you said, Paul. I mean, I did not say I have got an EK2 E. coli. I think I made that very precise. The reason I voted against the Woods Hole guidelines is because they did not stipulate that some person other than the person constructing it would certify that they felt the data were valid. So these data will be submitted, and they will be submitted for publication, and I might say that the preliminary results that were obtained some time ago have just come out, I guess, in the recent edition of NARSM. There are about 6 pages in there.

DR. BERG: I want to try to identify the procedure. The procedure in the guidelines states that the claim of the existence of an EK2 vector has to be verified by independent testing, and a whole series of testing.

DR. CURTISS: You mean EK3.

DR. BERG: No, EK2.

DR. HUTT: That is the point. I would like you to set the page that that procedure is laid out.

DR. BERG: You have to do laboratory tests to certify that that 10^{-8} containment is possible and can be done.

DR. CURTISS: Somebody else has to say that they believe that--

DR. BERG: The committee.

DR. CURTISS: The committee, right.

DR. HUTT: Is that spelled out?

DR. BERG: Yes.

MR. HUTT: It is spelled out that it will be given to other laboratories for verification.

DR. FREDRICKSON: Perhaps someone could identify the page and help us with the exact question.

MR. HUTT: I could not find that procedure spelled out, Dr. Berg.

DR. FREDRICKSON: Dr. Rowe, you had a point. You are a member of the committee, the NIH committee.

DR. ROWE: I want to just comment on a couple of aspects of it. I heard what you said, but I think the point between the technical committee and the policy committee is the difference between our DNA program advisory committee and this whole committee today. You are the policy committee, to decide on the broad base, the public interest basis of whether what we did technically is then adequate protection for the public. I think that structure is already there and that is why you are here today.

On the other thing of going ahead, and the question of whether the committee should have considered going slower, and so on, as a person who took a very conservative, that is a very restrictive position on the committee. I think we are in the very safe position in that what I think we did was a very great overkill in that we have built in conservatism on top of conservatism, and I think we have done a very good job of restraining or writing guidelines that are very restraining in relation to what will eventually be seen as the real magnitude of risks. I think we are not subject to accusations of having been cavalier at all in setting this thing up. I think we have really overkilled in the other direction, and have done a very safe job.

DR. WRIGHT: My comments were meant to apply in general rather than to the particular committee, except as far as the composition is concerned. So I am not sure that what you say answers really the point that the committee operated under the presumption that the research was acceptable.

In my view it would have been preferable had a first committee advised simply on the nature of the risks and how to contain it instead of--and then a second committee would then advise on whether that would be acceptable. As I see it, that distinction was not clearly drawn.

DR. FREDRICKSON: Our time is running a bit short, but I will recognize first Mr. Hutt and then Dr. Koshland, and then Mr. Ladwig.

MR. HUTT: The only sentence that I have been able to find is the first sentence on page 18, and Dr. Singer, I would hope you would correct me, but it says "EK2 host-vectors - These are host-vector systems that have been

genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory."

I think one could, as an independent scientist, in interpreting these data make his own judgment based upon his own laboratory results without being required to do the way this is presently written, through any advisory committee procedure whatever. Now, there may be additional requirements that I did not see.

DR. SINGER: I think there are a couple of places where more specific indications of the kinds of tests that should be done are given for systems using plasmid vectors.

MR. HUTT: Where are you reading from?

DR. SINGER: Okay, starting on the bottom of page 20, I guess.

DR. FREDRICKSON: This is in the draft guidelines in your brown book.

DR. SINGER: There is an indication of the kinds of tests that might be used with plasmids.

DR. HOGNESS: It is on page 25.

DR. FREDRICKSON: Dr. Hogness says it is on page 25.

DR. SINGER: Well, I was coming to that, yes.

Further on, the same kind of thing is given for the lambda system--that is, specified use of the lambda tests that might be prescribed--and then finally, at the top of page 25 is a more general statement no longer in italics.

MR. HUTT: But the top of page 25 is not a requirement, it is merely an encouragement. "Should" means you don't have to, and "is encouraged to" means you don't have to, so that your procedure that you have laid out is not mandatory. It is one that is encouraged.

DR. BERG: I am sorry, but was it not true at the end of the La Jolla meeting there was a motion made and approved by the committee that no EK2 vector would be certified for use by anybody, or accepted until the committee had certified it?

MR. HUTT: I just don't see that in here.

DR. FREDRICKSON: Would any of those who were here on the committee have any objection to those minutes entering themselves into the guidelines?

MR. HUTT: That would solve the problem.

DR. FREDRICKSON: Dr. Hogness?

DR. HOGNESS: You might say that in general the word "should" was used.

DR. FREDRICKSON: You mean because you were advisory? And you waited for Bethesda to put the articles-of-war language?

(Laughter.)

All right, then I think the sense of that is clarified.

Now, Dr. Koshland?

DR. WRIGHT: May I ask one question before we move on to another subject?

DR. FREDRICKSON: Yes, you may. Although your time is up, you may.

DR. WRIGHT: Pardon?

DR. FREDRICKSON: You may.

DR. WRIGHT: Thank you.

I am a little confused, but does what you are saying mean that all the tests are to be done prior to all experimentation, or will some work start before the tests are complete? What I am concerned about, obviously, is that the experimentation of E. coli should be rechecked, checked by others in the field before any experiments are carried out. I am not sure that is the case.

DR. FREDRICKSON: Dr. Berg?

DR. BERG: As I understand it, certification of EK2 vectors is necessary prior to its utilization in any experiment that calls for an EK2 vector.

DR. WRIGHT: Who will certify it?

DR. BERG: The committee, the committee which was set up and drafted the guidelines must review the documentation claiming an EK2 vector, and must actively certify it, and no experiments that require an EK2 vector can be done before that has been certified and released.

DR. FREDRICKSON: Dr. Koshland?

DR. KOSHLAND: I just want to say that I think at the moment, as an outsider to this area, that there are data that there is a possibility of a risk, and there are data that there is a possibility of benefit, but I don't think there are data to indicate the nature of the risk. We just don't know. There is no way of assessing it right at this moment in any way but a guessing game, and it seems to me, anyway, from all that has happened today, that I would be very interested in seeing the NIH or some other organization take on some of these risky experiments, perhaps at Fort Detrick under very careful conditions so that we have some data on which to make an assessment of risk.

I would also hope that the guidelines remain that. That is, that they remain in some way a flexible-enough instrument so that they could be rapidly--well, not rapidly, but at least more easily, changed than if they were firm regulations and so forth. I think you are going to have to have that kind of response in these guidelines to be responsible in this area.

MR. FREDRICKSON: Mr. Ladwig.

MR. LADWIG: I think Dr. Wright has brought up an interesting point on the decision-making process, and I don't think this was said today. If you want to hold it until the end of today's meeting, that is fine with me, but I think there should be a point that, number one, this is an advisory committee here, this isn't a policy making committee. If I am to understand right, we are just advising you and you have the responsibility, Dr. Fredrickson, of making the decision on accepting these guidelines, correct?

DR. FREDRICKSON: That is correct, Mr. Ladwig.

MR. LADWIG: Perhaps what we need is a clarification at the end of today as to what the process is going to be from here on. In other words, when will you make the decision? Are you under any kind of a deadline because of some kind of a Nobel Prize deadline?

(Laughter.)

DR. FREDRICKSON: I will address that issue tomorrow.

MR. LADWIG: Okay.

DR. FREDRICKSON: Now I think we had better move on. Thank you very much, Dr. Wright.

Dr. Brown, I would like to amend my statement to you. If you would like to file your variorum edition of this for the record, we would be very pleased to have it. You referred to something you have.

DR. BROWN: I have Xeroxed my comments like others, and put it over there. It is a partial variorum of the particularly outstanding areas that I disagree with. I could produce a full variorum edition, but I don't think you really want that.

(Laughter.)

DR. FREDRICKSON: I would certainly not deny you any privilege that I have already given Dr. Goldstein if you wanted to work on that tonight.

Now, Dr. McCarthy, all members of the committee will have received by the end of today the statements that have been filed?

DR. MC CARTHY: I think they have them already. The only one I do not have is Dr. Sedat, who told me that he will get his text into us several days after the meeting.

DR. FREDRICKSON: All right. He is not speaking until tomorrow, per his request.

DR. MC CARTHY: I don't expect to have his even by tomorrow.

DR. FREDRICKSON: Therefore we have a partial statement for the record and available to you of what Dr. Brown was earlier referring to, and now, it is his turn.

SPEAKER: We do not have Dr. Brown's statement.

DR. FREDRICKSON: Then we shall see that you get it--

(Administrative discussion)

DR. FREDRICKSON: Dr. Donald Brown is from the Carnegie Institution in Baltimore. Dr. Brown?

DR. BROWN: Thank you very much for the invitation to come down and participate. I have a number of things to say, and I couldn't possibly say them all in 10 minutes. I thought perhaps I would just limit my comments to three different areas. One is the nature of the hazard as I have seen it and some of my colleagues. Second is my own history of Asilomar, which agrees to some extent with the last speaker who was here. And finally, some views, which the last speaker also mentioned, of perhaps how this process of examining hazards might be dealt with in a more effective and--in a way which everyone would agree with in the future.

I am a biologist who has worked on gene purification for a number of years, and it was in fact the frog genes that we purified which were first

cloned and put into bacteria by the California group. I was, perhaps for this reason, asked by the Asilomar conference to assemble a group, and Dave Hogness was one member, to assess the hazards of putting animal and plant genes into bacteria.

Now, it seems to me that the one thing which was never discussed in any great detail, and I am very delighted to see the members of the advisory committee discuss it, was the nature of the hazard itself. There has been very little discussion of how serious the hazard is. Can we really assess this hazard?

The more I talked to my own colleagues, the more it seemed that the hazard was remote. It became more and more remote the more we talked. How can you assess a potential hazard? I personally do not believe it is a risk-versus-benefit operation.

In basic biological research it strikes me that the risks must be minimized. We cannot by all means even predict really the benefits. I think we can quote examples of that many times over.

But there really is a way, it seems to me, that you can kind of assess the hazards of this kind of research. You can compare it with the original source of the DNA. Now, this is a kind of exercise that I have subjected a number of virologists to, and I understand there are virologists, perhaps, on this committee, and Dr. Melnick, you might want to consider this little exercise.

Suppose you had an animal virus like SV40, one which is used under P2- and P3-type containment in most laboratories, just physical containment alone--2 guaranteed infectious particle. Members who work in the laboratories get their serum converted to SV40 positive. They weren't before. So it is infectious.

Now, supposing you had an important experiment in which you wanted to work with this viral DNA. Which would be safer? To grow large amounts of tissue culture material and purify large amounts of virus from this tissue culture material, guaranteed infectious particles, or to clone this genome the way you have heard it described, and grow up a liter of bacteria and get about the same amount of DNA from this liter of bacteria.

Now, how do the two hazards match up? One, we have got the guaranteed infectious SV40 particle. Two, we have recombinant DNA with that virus in it. Now, what do you have to do to be poisoned by this recombinant DNA? I might add that as we all know from the guidelines you cannot do the second experiment now. There is a moratorium against it. You can't even use it under the same containment that you can work with SV40. It is illegal. You can't do it.

However, what is the way in which you would be poisoned? First you would have to drink down a large amount of this recombinant DNA, this bacteria containing recombinant DNA. This bacteria would presumably have to colonize or transform or transfer its infectious plasmid containing the thing to other bacteria. It would have, to a certain extent, to colonize.

Now we have heard all about 10^{10} this and so forth, but that is where the discussion in hazards has stopped. But that is not the end of the biological containment which exists in humans, because having colonized the gut, the bacteria has to break down and liberate these poisonous plasmids. This naked DNA material must withstand the hostile environment of the gut. This naked DNA isn't even finished yet. It still has to go inside of the gut cells to give you an infection, and what is the final infection you get? It is SV40, which you could have gotten very nicely with the original infectious virus to begin with.

Now, you will say to me no, look, you can get changed types of infections with a large amount of material. There are all kinds of potential hazards here and there. I have worked with SV40 for a long time and it hasn't killed a lot of people.

I maintain that it is now believed that SV40 is a bit more dangerous than it used to be thought. There are, therefore, potential hazards in working with the original source material.

I consider it legitimate, then, to compare the new method with the old method.

Now, it is now illegitimate to shotgun all vertebrate DNAs, and that goes all the way from man down to fish. You cannot shotgun. There is a moratorium on this. What are the hazards in this DNA that we are afraid of? You have not heard much of a discussion of these hazards because they cannot really be defined.

Now, generally the way the guidelines are constructed, these hazards are usually infectious agents, viruses. Why, because you can use embryonic DNA or primary tissue DNA rather than the adult. That suggests that the framers of these guidelines must have had viruses in their minds.

And yet, the chances of picking one of these up, the chances of going through these three or four different biological containment steps are exceedingly rare, as I have pointed out.

Now, I want to give you some specific ways which I believe that these guidelines could be made very strict in containment, far stricter than I frankly believe is legitimate, and still be flexible, because you do realize how rigid these guidelines are.

The word "guidelines," by the way, is a terrible word for this. These are not guidelines. These are rules. Rigid rules. EK2 doesn't exist, therefore these experiments are out. Let's not talk about guidelines. These are rules.

Let me give you one example of how the manner in which an experiment is done might be considered. It is now possible, courtesy of Dave Hogness and his collaborators, to shotgun DNA, put it on plates, and never ever have a liquid colony of those potential hazards. That is to say the DNA recombinants that you don't want will never go beyond about 10^8 . That is the concentration of one colony on a plate, no liquid cultures, no nothing. All of the final steps of selection are done just on that plate and nowhere else. No liquid cultures. There is no pipetting, so you can't even ingest the stuff.

Now, it seems to me that the manner of experimentation should have been considered in these guidelines. There are safe ways of doing it. You can't even take these eukaryotic DNAs and go to Fort Detrick in these P4 containment rooms, have some very well-trained assistant do the shotgun procedures for you, select on plates, the way I just said, the colony that you want, and take it back to your laboratory, because it is illegal. That is to say, they are so totally inflexible--these rules are, and you can't even use P4-containment rules in their present way.

Now, that is the first thing and I probably used most of my time up but I would like to agree with the previous speaker that there is a certain civics lesson to have been gained from the way in which these decisions were made. We happen to--our final conclusions are different, but the general principles to begin with are the same.

It seems to me that the present committee, the NIH guideline committee, and before it the Asilomar committee, were really handicapped because of this self-serving issue. What happened was that when scientists brought up the issue of potential danger, an issue which was singularly singled out free of all other issues, and put out, when they brought it up they got some points. But when they tried to control it, that is when the trouble began.

I would like to suggest that perhaps a useful thing for this committee to think about is the way in which a completely objective--a committee which cannot be accused of self-serving, a committee which does not have members whose minds are made up in either direction ahead of time--can be formed,

because I really believe that sooner or later there are going to be other hazards singled out by the biological community for assessment, and they might in fact even be real hazards that are singled out.

(Laughter.)

I think there should be a way in which a mechanism by which these can be brought to the attention and treated in a jury type fashion. I wish to point out to you that neither at Asilomar nor at the meetings of this NIH committee has there ever been a hearing of testimony. No one has ever invited me to come and give my two cents in a testimony-type fashion. I have been invited to meetings to sit on the outside, and there is always this self-serving thing which continually is a troublesome problem for all of us working in the area who want to have our opinions known.

But I really think that there is a future to this type of regulation which you ought to address yourself to. How can this committee really point out, really have a truly objective way of assessing this hazard and future hazards?

Now, what else do I want to say? I have probably run out of time.

DR. FREDRICKSON: Yes, you have, almost.

DR. BROWN: I am sorry. Well, maybe I can answer questions, then, rather than haranguing further.

DR. FREDRICKSON: I gather from your statement that you had some specific objections and recommendations, and the committee will have those before them.

DR. BROWN: Well, you will see on this typewritten thing that the last two pages deal with -- are entitled "Specific Objections to and Recommendations for the NIH Guidelines."

DR. FREDRICKSON: Right.

DR. BROWN: These deal with my major objections. Some are fairly technical which I will be glad to answer if you would like me to. Features such as purifying DNA 99 percent pure. I doubt that any member of the committee can tell me how that can be done and judged. I would like to hear a member of the committee tell me. You might as well just say they are banned.

DR. FREDRICKSON: Dr. Singer?

DR. SINGER: Oh, I am sorry, I was waiting for your call for comments.

DR. FREDRICKSON: Well, in effect I did.

DR. SINGER: In terms of just responding to one of the points that Dr. Brown made, which is the question of the flexibility that is built into the guidelines in regard to physical containment. The guidelines specifically recognize the very miniaturization procedures that you describe.

DR. BROWN: They state it and then they do nothing with it.

DR. SINGER: No.

DR. BROWN: Then how does it affect the guidelines, Maxine?

DR. SINGER: It affects it in the following way. Let me put it the way I was going to put it.

First of all, on page 3, at the end of the introduction, the guidelines recognized that there will be new and special procedures which will allow experiments "to be carrier out under different conditions than indicated here without sacrifice of safety." And then again, on the botton of page 6, at the end of the discussion of physical containment, it specifically recognizes the procedures developed in Dave Hogness' lab as offering a new procedure that allows the containment of recombinant DNA-containing organisms. I think those two recognitions, specific recognitions, coupled with the fact that the guidelines are required to be reconsidered at a least yearly intervals, seems to me at least to suggest that the committee was aware that such new procedures that offered new ways for containment are being developed and present useful and important alternatives to the specific procedures that are in here.

So I just really meant to point out that I think in some sense the guidelines respond to this.

DR. BROWN: The guidelines say that here is a new method which may be useful, but it does not give any reduction in stringency because of the existence of this new methodology. I would like to see written in the guidelines that you could bring everything down one notch in physical or EK containment if this particular type of methodology is used.

DR. SINGER: At the bottom of page 6, there is the following statement, "Thus, such procedures should reduce the need for the standard types of physical containment."

As you know, I am not on the advisory committee, but I was at a number of their meetings, and certainly in terms of the discussion that went into that paragraph and that sentence, I think there was very much in mind exactly the point that you made.

DR. BROWN: Can I ask you and Paul a direct question? Would you advise that these guidelines should stipulate that, using this new methodology, that physical or EK regulations can be reduced by one notch?

DR. SINGER: I would certainly hope that with an evaluation of those methods, that precisely that sort of thing would happen.

DR. BROWN: What kind of evaluation would you have in mind?

DR. FREDRICKSON: Dr. Berg would reply to that cross-examination.

DR. BERG: Only on technical grounds. Using that technique of screening, you know what you can do is you can work with micro-colonies which, as you say, you need not pipette, and you can screen large numbers to look for a clone which contains a sequence that you want.

It may also, and often will, contain sequences that you know nothing about.

DR. BROWN: Along with the one you want.

DR. BERG: Yes, along with the one you want.

The second step is that you now have to pick the clone which you have identified and you must grow it up in order to get the DNA you want. So it is not now--it is passed from a micro-colony on a filter to something where you have to grow it up and culture it, centrifuge it, collect it, and so on.

DR. BROWN: Paul, may I respond?

DR. BERG: No, let me finish.

DR. BROWN: I am sorry.

DR. BERG: All the procedures which have been known to, in fact, generate the kinds of aerosols and possible contaminations that we know we are talking about.

So the fact that you have identified the clone on a filter has not ended the experiment at that point.

DR. BROWN: Now, can you explain to me, then, how one would ever characterize a clone?

DR. FREDRICKSON: I wonder if we are not bogging down, Dr. Brown. I don't want to interrupt that line, but I want to go in order.

Dr. Melnick, did you have a point relative to this?

DR. MELNICK: Yes. Dr. Brown raised the question as to whether working with the virus itself might not be more dangerous than working with the DNA recombinant. And while this may be true, it may be more dangerous to the individual worker, it will not--SV40 will not spread from person to person, whereas if it gets into an E. coli and is let loose in the community, theoretically it may be spread from those people infected with E. coli.

DR. BROWN: It is not clear to me how you say that, Dr. Melnick. It is my understanding that workers in a laboratory get infected with SV40.

DR. MELNICK: When they are working with the virus itself, but they do not get infected from their neighbor.

DR. BROWN: You mean then they don't go back to their family and re-infect them? It should be stated in the same tone then, sir, that it has already been stated by Roy Curtiss and others that the E. coli K12 does not multiply in the gut. It just goes through the gut, and therefore it strikes me as very bizarre that if such a bacteria which does not multiply in the gut is able to produce any kind of epidemic--

DR. MELNICK: Well, I think the data that Roy Curtiss showed us today, that E. coli would probably be harmless also. I don't think there would be the risk.

I was just answering your question on this virus and the potential danger of having recombinant DNA.

DR. FREDRICKSON: Dr. Hogness?

DR. HOGNESS: Well, I only wanted to quote to Don and re-emphasize what Maxine said to Don in relation to page 3, which is that "Indeed, we urge that individual investigators devise simpler and more effective containment procedures, and that study sections give consideration to such devise simpler and more effective containment procedures, and that study sections give consideration to such procedures which may allow change in the containment levels recommended here."

We put that in, anticipating that you would want to make a case for lowering something from P3 to P2 because such and such might include a new recombinant procedure. You make the case to your study section, and we are encouraging them to consider such flexibility.

DR. BROWN: My question is whether or not these rules, under these rules, it is possible to reduce for example EK2 to EK1.

DR. FREDRICKSON: Mr. Hutt?

MR. HUTT: Dr. Brown, as I understand you, it seems to me that there are two possibilities here. One would be in terms of the flexibility we desire and that Dr. Koshland mentioned a short while before. One would be in a sense an ad hoc variation from the rules by an individual laboratory for good reason, that they advance and which might be agreed to by the advisory committee.

The other is for amendment of the rules that would apply across the board there. Now, would you feel more comfortable and others in the room who are concerned about the flexibility issue, if procedures were incorporated in the rules, and I agree with you, they are rules, not just guidelines, that would specifically provide a mechanism for that to happen?

DR. BROWN: Oh, by all means. I much prefer the notion that I as an experimentalist can go to some committee, present my physical, ecological containment experiments I wish to do, and have them judge it individually rather than have some blanket ruling irrespective of the manner in which I do my experiments. I much prefer that kind of arrangement.

Why, even in the human experimentalist category they are premitted to give an airing to their experiments.

DR. FREDRICKSON: Thank you very much, Dr. Brown.

Dr. Silverstone, you still have five minutes remaining of your original time. Did you wish to use it?

(Laughter.)

Well, five and a half. Dr. Silverstone is from MIT, as he reminded me earlier.

DR. SILVERSTONE: I hope the committee's endurance is as good as our latest cross-country ski winner, because it has been a long afternoon.

I am a member and participant of several groups, including the recombinant DNA group of the Boston Area. The other groups were the Genetics and Society Group of Science for the People, which sent you a mailing concerning certain other issues, and a group at MIT, an ad hoc group of biological workers that have formed a health and safety committee. It is composed of technicians and laboratory assistants, representatives of the trade unions including the custodial unions, students, post-docs, and faculty members.

The concern of these groups is not abstract with these guidelines. The members of these groups are among the first to be exposed, and like

workers in vinyl chloride plants, are the guinea pigs for the safety or lack of safety of the new technology.

The danger of these agents, as we have been describing, is unique in that they can be spread like a bacteria infection, and unless it can be assured that the possibility of danger is reduced to insignificance, we would suggest that NIH must withhold funding until that level of safety can be at least established.

We do not propose this lightly. We recognize that many scientists wish to do these experiments, and thus far the restraint has been admirable.

In all facilities from P1 to the P4 level there will be a distinct possibility of cross-contamination of cultures--bacterial cultures and cell cultures--and even the most expert microbiologist using the most sophisticated equipment will contaminate a culture. Whether the agent of contamination is E. coli or mycoplasma or what have you, the danger is clear. No concept of safety within a facility can be centered on one experiment or on one experimenter.

The possibility of multiple interaction is likely and should be weighed in establishing safety guidelines.

Our main concern has been in the area of the mechanics of regulation, and I hope you will read our statements. We feel the La Jolla guidelines exempt institutions from responsibility and put all of the load on the principal investigator. We do not agree. We think that the institution must be equally liable, especially in terms of legal suits that the employees or communities might bring for experiments gone awry.

We have too many recent examples of unscrupulous investigators in biomedical science to be sanguine about this issue. Victims of error in these types of experiments should be able to extract financial compensation from both the investigator and the institution if safety guarantees or procedures are violated. This creates some pressure on the institution to protect custodial and support help as well as other workers who normally do not interact with the principal investigator.

The abuse of these relations at MIT is unfortunate. Our local ad hoc safety committee has heard more tales than are worth recounting to you, custodians stabbed by uncapped needles, improperly disposed of broken glassware and so forth. These are laboratories that have been dealing in growing viruses that are known to cause cancer in mammals.

In a survey which we handed out to 400 workers in the MIT Biology Department and Cancer Center, we received 130 replies. Of these, more than 75 percent were not given pre-employment physicals, or physical examinations in the past two years. More than 80 percent ate or smoked

or saw such incidents in areas where it was prohibited. More than 80 percent did not know who the safety coordinator was. I have further examples which I will submit to the record tomorrow.

I know our local unofficial committee's efforts in conjunction with renewed concern by the MIT administration will have a profound effect in changing such practice, but I think that without the active participation of students, technicians, laboratory assistants, custodians and even secretaries, in addition to principal investigators, many of whom are not regularly in the lab during the course of the day, there can be no real safety practice or enforcement of safety guidelines. That is, we argue strongly for local rank-and-file safety committees who would be able to lodge protest to both the institution and the funding agencies if safety criteria are violated.

Finally, there is also the question of dissuasion and, if I might be so strong, reprisal for those who violate or wish to violate the recommended procedures. I do not wish to suggest the necessity of some kind of Mafia to engage in the protection business; however, without making some concrete proposals, the La Jolla guidelines leave everything to the essential goodwill of the principal investigators.

In a time when society is recognizing the need for more stringent regulation of environmental carcinogens and other health hazards, when the Occupational Safety and Health Administration is struggling to deal with the regulation of these agents in the workplace, we should set an example rather than join a past practice that has only too clearly caused unnecessary illness and suffering. Thus, I think we must suggest the possibility of cutting the funding of health and safety violators and rejecting the publication of experiments that do not observe safety practice, although perhaps publication and public censure would be better. We believe it is inappropriate for the peer review process to become tied up in the question of evaluating safety. We do not believe peers in molecular biology are the best or sole judges of safety on a technical level. We would rather see a committee that evaluated, separately from scientific merit, the proposed safety features of an experiment.

This committee, a biohazard review committee, would include--in addition to the molecular biologists--epidemiologists, occupational hygiene specialists, ecologists, and others capable of evaluating of a potential public health hazard. I feel these people should be in the majority on such a committee.

I am not opposing peer review to judge scientific merit, and hopefully such a biohazard committee would function more to educate the investigator rather than to censure her or him. However, I would urge you to

read our arguments against peer review in this area in our extended statements, and measure this against what the La Jolla guidelines are suggesting. Thank you.

DR. FREDRICKSON: Thank you, Dr. Silverstone.

DR. MC CARTHY: May I interrupt just for a second, please? Additional copies of the Boston statement have just arrived, so we now have about 250 of them.

DR. FREDRICKSON: We have about 4 minutes for discussion and comments or questions for Dr. Silverstone.

Mr. Hutt?

MR. HUTT: Could I just ask what you would propose in lieu of guidelines? What form of regulatory control are you proposing?

DR. SILVERSTONE: Well, we aren't opposing the existence of guidelines. I wouldn't go along with Dr. Brown.

MR. HUTT: Then in addition?

DR. SILVERSTONE: In addition to guidelines, which I think would reflect general scientific thinking and general evaluation, we would recommend both local institutional biohazard review committees that would have certain authority, that would be able to criticize without the fear of lost jobs, and we are suggesting that these be composed of more than just principal investigators.

We also propose that there be an independent body in the grant review structure of NIH that would consider the question of safety from a public health standpoint.

DR. FREDRICKSON: Yes, Dan?

MR. SINGER: There is one way in which one might catalyze activity at host research institutions, and focus concern on the questions that have been raised so well, I think by Dr. Silverstone. Let me suggest that were there to be issued by NIH as part of the guidelines, or in supplement to the guidelines, a requirement that in addition to the host institution's certification of the availability of particular containment facilities, the host institution should also be required to certify the availability of compensation within that host institution for anyone injured in the course of the experimental procedure, I suspect that the ripple effect of that kind of certification requirement would tend, in a very drastic and quick way, to focus attention on precisely the kinds of issues that have been raised here.

The institutions themselves might be free to work out, in recognition of peculiarly local conditions, the particular mechanisms for compensation. It is not simply enough to be safe in a dangerous world, or to try to be safe. Where there are going to be injuries, those injuries have to be compensated.

DR. FREDRICKSON: Thank you. Those comments were from Mr. Dan Singer.

I think that I have time for one more comment. Dr. Shaw?

DR. SHAW: In followup here, I think that the ripple effect might extend to human subjects in any experimentation, and if we are going to require informed consent by the institutional review committees for human experiments, then I think that we should consider the need for informed consent for laboratory workers, for janitors, for secretaries in these areas of biohazard, and that this could well be considered in tomorrow's discussion of procedures.

DR. FREDRICKSON: Thank you, Dr. Shaw.

The last public witness that we will hear today is Dr. Steven Wiesenfeld, from the Jewish Hospital in Denver.

Yes, Dr. Redford?

DR. REDFORD: I just wanted to add the comment to the discussion we were just having, that it seems to me that part of the question really raised is not whether we should have peer group committees, but their composition. This would be a question for the NIH as to what directives it gave the institution with respect to the composition of peer group committees. That would be a very important administrative consideration.

With respect to insurance as an incentive to institutions, I suppose that would depend on who was paying for the insurance. In my institution, all liability insurance is paid for by a fee collected from the faculty. I don't think that would have an effect upon the institution.

DR. FREDRICKSON: Thank you.

Dr. Wiesenfeld?

DR. WIESENFELD: Thank you, Dr. Fredrickson.

I come before you as a spectator and I hope that my comments are helpful to the committee. I represent only myself and no particular group. I am a physician at the National Jewish Hospital in Denver, and I am trained in internal medicine and immunology, and I spent two years at NIH in the slow virus laboratory of Dr. Gajdusek and Dr. Gibbs.

After two years of grinding human brains from transmissible virus dementias, I developed an intense personal interest in biosafety, especially since it takes probably 4 to 20 years to determine whether I am going to come down with my own slow virus dementia.

I think that people both in and out of the scientific community owe a debt of gratitude and perhaps much more to the members of the original committee for having the courage to call this moratorium, and I think that the period has been very healthy for science.

However, there have been a lot of polarities of view, and at certain points it seemed that the dialogue that was going on had almost ceased, and I think there is a great danger in this. I think the danger is that it will lead to a lack of moderating influences on a particular event. So I would strongly urge that we keep communication channels open, especially between divergent groups, and perhaps even have these channels formalized through NIH and have meetings such as today's if people can survive them this long.

My specific concerns about the guidelines again are meant to be constructive. Containment guidelines for the shotgun experiments for different classes of animals are uneven. We are only dealing with hypothetical hazards, but there is no way of judging whether or not shotgunning invertebrate DNA is any less risky than shotgunning non-human primate DNA.

With regard to the cold-blooded vertebrates, they can be shotgunned with EK2 plus P2, and embryonic tissue from cold-blooded vertebrates can be shotgunned using EK1 plus P2.

Now, the proposed dangers of recombinants using embryonic tissue seems to me to be equal to the dangers of using adult tissue, especially with regard to oncogenes, hormones, and enzymes. I would think that maximum potential risk should be assumed until we have some evidence to the contrary, and I would urge that all shotgun experiments be considered of equal risk. I would urge the committee to adopt a very conservative attitude as to what the confinement should be.

I would like to make some comments and add some data about physical containment. I don't think we should put too much reliance on physical containment, and I don't think we should rely on anecdotal reporting of laboratory safety. The fact is that most labs have not and do not follow their personnel. Reviewing the data in the literature, P1 and P2 physical containment do not provide probably any significant protection. P3 containment involving laminar flow hoods is also far from fool-proof, and there have been seroconversions to SV40 in P3 labs. At least one third of the 5,000 laboratory-acquired infections in the last 30 years have occurred in P3 facilities. This data comes from Science in December of last year. I know that number, 5,000, is a little different from the number we heard today.

Under P4 conditions at Fort Detrick, there were 423 cases of infection and three deaths in 25 years. Dr. Wedum, the former biosafety officer at Fort Detrick, has simply stated that all researchers are probably exposed to significant quantities of the agents they work with.

So what about more recent experiences with P3 facilities? With newer P3 facilities, such as those at the CDC, there were four known lab-acquired infections in 100 workers from 1960 to 1967, or 0.006 cases per man-year. This data comes from Dr. Kissling, and it is published in Biohazards in Biological Research, and I see some of you have that book.

I talked last week to Dr. John Richardson, who is the director of the Office of Biosafety at CDC, and he has reassayed the hazards and relooked at the data that Dr. Kissling originally looked at and found that there was gross underreporting and probably the number of laboratory-acquired infections is probably on the order of tenfold greater.

They have had, however, no known infections in P4 sealed-glove cabinets. These cabinets, however, have been known to explode, and the most dramatic example is that of the cabinets that contained the moon rocks.

Dr. Richardson feels that any type of safety cabinet is at best an adjunct to good techniques. I think that is what we should be stressing here. Even at such excellent facilities as Building 41 at NIH, uncontrollable vectors such as cockroaches have been found within the high containment areas, and experimental animals have escaped from maximum to lower containment zones.

There is one other area I would like to mention just briefly. There are other existing or potential recombinants, and I include here DNA recombinants as well as RNA recombinants not covered by these proposed guidelines. Whether they could be included by this committee or by a separate committee, I can't say. They may present more of a biological hazard than the recombinants that we have been discussing today for two reasons. One, the recombinants result in a stable genome versus the insertion of foreign excessive genes into E. coli, and two, the investigators who are working with the recombinants I am going to mention may be less aware of the potential biohazards.

I can only list examples. The first is recombinant influenza viruses for the purposes of obtaining vaccines. The second is experimental and naturally occurring SV40 adenovirus recombinants and experimental SV40 reovirus recombinants. The third includes attempts to transform human tissue from slow virus diseases and tumors using SV40 to produce long-term cultures.

Incidentally, anybody who has worked in the tissue culture lab knows that there are frequently contaminations of tissue cultures with bacteria, and a naturally occurring SV40 recombinant could potentially insert itself into E. coli, and unbeknownst to the researcher the experiment we have been trying to avoid could well happen.

The fourth area is inserting tissues from human transmissible diseases into animals known to have similar naturally occurring diseases. An example of this is human hepatitis. Human hepatitis A virus has been transmitted in primates known to have their own form of hepatitis virus. A naturally occurring human-animal recombinant hepatitis virus is always a possibility. It could well be stable, novel, and uniquely pathogenic.

Another example is systemic lupis erythematosus, which is at this point a candidate virus disease in man. Transmissible agents have been demonstrated in certain animal species for SLE, and putting SLE tissue into these species could allow for a unique recombinant SLE.

I think I will stop at that point. I would just recommend that the committee consider these area, and also I would urge a very conservative attitude on these experiments. Benefits may come in time, but first I think we must prove to our own satisfaction that we are going to do no harm.

DR. FREDRICKSON: Thank you, Dr. Wiesenfeld. We have four minutes for any final questions or discussion on Dr. Wiesenfeld comments. If not from the committee, I see a hand back there.

Yes, Mr. Madansky?

MR. MADANSKY: I would just like to add one other area in which possible recombination can take place, which perhaps the committee hadn't thought of or hadn't considered. That is that area which would involve doing experiments under low containment facilities or none at all, but doing unrelated experiments in the same lab. For instance, doing restriction mapping of animal viruses in one corner of the lab, and then doing a shotgun of any kind of experiment in cloning on an invertebrate in the other corner of the lab. Possibly, and apparently it has been known to happen, you can get one piece that shouldn't join into the invertebrate DNA, and get cloned. Therefore, you have a situation where you really should have had containment facilities much higher because two unrelated things go on in the same laboratory.

DR. FREDRICKSON: Thank you.

Dr. Barkley, did you have a comment?

DR. BARKELY: Yes, I asked Dr. Wedum if he would kindly prepare a historical review of the approximately 400 and some infections that were

recorded at Fort Detrick. He has done that, and I think it is of importance to point out that as they moved into the glove-box systems, their infections were almost diminished to nothing. I think they had one infection.

I don't care to take any time on that, but since the point was raised, I do have this report and would like to submit it to the committee for their review on this very elaborate discussion of the experience since 1943, at which time biological activities were being conducted at Fort Detrick.

[See Appendix B to these Proceedings]

Another point I would like to make is that Building 41, although it is and was designed as a P4 facility, and does possess those physical attributes of a facility, has not been operated as a P4 facility, because the hazards of the studies that are currently being conducted there have not been judged to need that sophistication.

DR. FREDRICKSON: That accounts for the cockroaches.

(Laughter.)

Dr. Nightingale?

DR. NIGHTINGALE: I have a comment on the guidelines with respect to the lower eukaryote shotgun experiments. It has come up before, but it is still not clear in my mind first, why things such as insects should be considered lower, which most taxonomists would not agree with. They are different, but they are not lower, and their ability to adapt and to change to differing environmental conditions is very marked.

This big category under invertebrates includes 14 or 16 phyla, and it is a very, very diverse category of living organisms, many of which are vectors of human, animal and plant diseases, and all of these vectors are known to man as being vectors. Therefore, the point I am trying to raise again because I still don't understand, is the inconsistency in the requirements between cold-blooded vertebrates and all other eukaryotes, because as Dr. Brown pointed out, P2 plus EK2 effectively precludes experiments with cold-blooded vertebrates, but P2 plus EK1 permits experiments with other cold-blooded animals, which includes all the insects and all the arthropods. That is one item.

The other is that when you look to the survival of, for example E. coli in the gastrointestinal tract, it is not the same as considering what happens to all the DNA released from the coli and its plasmid and anything else in the bacteria. I don't know about E. coli exactly, but for pneumococcal DNA at least, there is survival of soluble DNA in genetically active form for at least 48 hours in the mouse, and this DNA can effect genetic transformations

at a widely different site from the site of infection. Therefore, what should be looked for is survival of the genetic activity of a DNA fragment, not the E. coli per se.

DR. FREDRICKSON: We will ask Dr. Stetten or some member of the committee perhaps to comment on those two points tomorrow, Dr. Nightingale. Thank you.

When we convene tomorrow morning we have still two public witnesses to hear, and then Dr. Stetten, if he is able, or if anyone is able, to provide a summary of our discussion at this point, they will do so, and then we will move on for more interchange between the committee itself and the public toward the point where the committee members will be asked to provide their opinions and recommendations as we move toward the close of the morning.

This committee is now in adjournment until 9:00 a.m. sharp, tomorrow morning.

[Thereupon, at 5:35 p.m., February 9, the meeting was adjourned, to reconvene at 9:00 a.m. the following day.]

February 10 transcript.

DR. FREDRICKSON: We will resume our meeting with testimony from public witnesses. The number has expanded slightly from my last evening's compilation.

First we will hear from Dr. Zimmerman from the Environmental Defense Fund, and Dr. Zimmerman has agreed that he might be able to make this statement in three minutes.

DR. ZIMMERMAN: Well, I will see what I can do in this limited amount of time.

I am Burke Zimmerman, a staff molecular biologist with the Smithsonian Institution Radiation Biology Lab in Rockville, here representing the Environmental Defense Fund. Most of you should have a copy of our handout.

There are some points, three and four, which deal specifically with the guidelines, which I think are non-trivial. However, in three minutes I am not going to have time to go into those.

DR. FREDRICKSON: That has been circulated.

DR. ZIMMERMAN: Okay, thank you, Dr. Fredrickson.

What I would like to bring up are the first two points on this paper which really have not been touched upon. Number one, the thing that concerns me is not the legitimate scientific inquiry being done by NIH-supported scientists in this audience, but rather those from other sectors of the so-called scientific community which are beyond any jurisdiction that NIH would have. Particularly, what about drug companies who may have less noble motives than the scientists, and if under the pressure to make some biological which may have a profit value, they may well, even though they have verbally said they would comply with the guidelines, ignore them in the heat of competition with their competitors.

Another thing that concerns me is what about the Department of Defense. There is still millions of dollars being appropriated for chemical and biological warfare research. Now, while there is a moratorium that has been observed by DoD on the development of so-called offensive biological weapons, must we merely accept that assurance of DoD that this type of experiment will not be undertaken as our only guarantee for safety? That is, the technology now exists for doing downright diabolical genetic engineering experiments. Therefore, I wish to raise the question particularly to the lawyers present, and perhaps Mr. Hutt would like to comment later on this, what legal authority exists for extending these guidelines to all sectors of the scientific community, and if not, what can be done on this score?

The other point which I wish to discuss, the second point, is namely assuming that effective guidelines are defined and there is some mechanism for extending the guidelines to all scientists, how may they be effectively implemented and enforced?

Now, an analogy might be drawn with the handling of radioactive materials, which are supposedly under strict Federal control. Most of us who have been working in molecular biology or biochemistry labs have probably seen many blatant abuses by people who should know better in the handling and disposal of radioactive material. In many cases, technicians and lesser personnel are not trained in the proper technique. In many cases even senior scientists are not, and when they are, simply choose to ignore those standards.

I think there is an element of human nature that has to be dealt with, namely if a hazard can't be seen or felt or smelled, it is much easier to ignore than something that smells bad, for example.

What, if any, mechanism exists or can be made to exist that will effectively control the human error? I don't know the answer to this, but I really think it is a germane topic for this group to consider today.

I will be happy to answer any other questions on the other points raised on the guidelines. I think my time is running short. Thank you.

DR. FREDRICKSON: Dr. Zimmerman, the question that you raised initially of the force of the regulations and the nature of our consideration will be taken up a little bit later this morning, and I think I will ask the committee to reserve further comment on that.

If there are no urgent further questions for Dr. Zimmerman, then--thank you very much, Dr. Zimmerman. Your statement has been circulated and filed with us.

I will proceed then to call upon the next witness, Dr. Sedat, Dr. John Sedat, from Yale University. Is that correct, Dr. Sedat?

DR. SEDAT: Yes, it is.

One tends to view this process actually in the here and now. This is 1976, and we have a considerable amount of contemporary history of science to reflect on. For example, our reaction to nuclear power is quite different now than it was in the early 40's, for example, and for example, we consider plutonium as a very extremely dangerous substance, and it doesn't even replicate.

When you tend to reflect on this, one becomes sensitized to many issues that are actually hidden under the table. Let me give you some concrete examples.

Most molecular biologists consider that the ultimate equalizer is the autoclave. You know, you want to kill everything, you toss it in the autoclave and you can forget about it. However, when you start to reflect upon this, the DNA may not actually be killed by the autoclave, and we know, for example, the physical properties of the DNA in the cell, because it is topologically, covalently closed, may snap right back, and then when it gets tossed out down the drain, can easily infect the cell. You may knock it down a few logs or many logs, but when you consider you will have one liter of an infected culture, there are 10^{14} molecules there in that order, and that is a very large number. In any event, the molecules could easily be repaired in a recipient cell and the whole process started all over again.

The other point is, you might well have two innocent systems in the lab going on. For example, one person might be just mapping a tumor virus with these same enzymes used to do the DNA recombination process, and in another end of the lab someone will be doing a simple bacterial DNA recombination

experiment. Both P1 conditions. But because they are in the same lab and they use the same centrifuge and the same common equipment, and there are always these problems of aerosols, you automatically produce a very hazardous situation.

When we consider these kinds of things we have to ask ourselves very seriously what are the benefits of these things. For example, can we take any risk of letting the population accidentally have a bug that will produce, say, insulin in their intestinal flora. This is cited as a benefit, but you know, what really would happen if the population would have such a thing?

When we say, for example, let us produce more fertilizer by hooking these genes on to the root nodules, there are many other considerations. I mean, what would happen to weeds, for example, and what would happen if some of these genes started churning out interesting atmospheric by-products.

In retrospect, really the only benefits may be scientific. However, even these are somewhat ambiguous. We have not exhausted all of the imagination and scientific effort today. For example, one of the main scientific benefits comes when you want to amplify a piece of DNA, you want to find out which genes are on each side of the fragment you are studying, and on the controlling element, and people cite cloning as the only way to do this. But in fact, on the horizon, and in fact doable right now, this can be done to a certain extent in the test tube. There are DNA replication systems, for example at Stanford and for example at Princeton when you can amplify 10^2 , 10^3 times the actual starting amount of DNA. It is not hard to imagine extending this and being able to do everything in the test tube without resorting to bacterial systems.

I mean, we have to reflect on these kinds of possibilities, and because we can always increase the sensitivity of detecting these systems, and thinking of new ways of doing it, maybe we can do it directly without resorting to bacterial systems.

Where do we go from here? I would seriously ask the committee to recommend just simply delaying a lot of these experiments, and substitute in their stead experiments to ask questions about the hazards. For example, the question of going on at Fort Detrick, in which people ask whether there is a chance to seroconvert with these plasmids in the experiments. Just ask what are the risks, and delay the experiments.

We all know that this conference here, this committee, whatever it votes will have international consequences, and if it votes yes, there will be an exponential increase in the number of labs and the people doing these experiments. That merits serious thought.

Just seriously, what is the rush to do these experiments? We have several billion years of evolution behind us. Who is to say what we should just march in and start changing these things?

Thank you.

DR. FREDRICKSON: Thank you, Dr. Sedat. Would you like to remain there at the podium?

Are there any questions?

Dr. Melnick?

DR. MELNICK: I was at Yale for 20 years and used the autoclaves regularly. Do you have any evidence that any functional DNA has even come out of a properly autoclaved experiment?

DR. SEDAT: I have asked people that question. Now, many people were in a position to either have done the experiment or know the answer, and the answer, as far as I can tell, is that no one knows. It is a very interesting experiment.

DR. BERG: The experiment has been done. Ron Davis has done it.

DR. SEDAT: Did they start with 10^{14} molecules as a standard?

DR. BERG: I can't tell you it starts with 10^{14} molecules, but the feeling was that the DNA was utterly fragmented by the ordinary autoclaving procedures, circular DNA or linear DNA.

DR. SEDAT: And they couldn't be rescued or anything?

DR. BERG: I didn't do the experiment myself. I am just saying that certainly the experiment has been done, and I am sure I can document it for the committee and get the data if it is needed.

DR. FREDRICKSON: We would appreciate that, Dr. Berg.

Dr. Leder?

DR. LEDER: With regard to the production of insulin in the intestinal tract, I think as every diabetic knows, much to his displeasure and discomfort, it has to be injected in order to be pharmacologically effective.

DR. SEDAT: Well, I mean, we really don't know what would happen if it would be produced right at the juncture of the epithelial cells.

DR. FREDRICKSON: I wonder if any member of the technical experts who are here would care to enlighten me a little bit further about Dr. Sedat's comment that these experiments that we are proposing guidelines for need not be done, that information could be obtained in a different way. Dr. Berg?

DR. BERG: Unless John has some understanding or insight that I lack here, Arthur Kornberg's system for replicating DNA will replicate whatever you put in the test tube. Therefore it is necessary to start with something that is pure in order to amplify that component. So if you take the total DNA of a mouse you will make more of the total DNA of a mouse, which is no big trick because you can isolate as much DNA as you want.

But if you are asking if you want to isolate a specific segment and produce large quantities of a specific segment that occurs with a frequency of one part per million, there is no way, using in vitro DNA replication that I know of, for doing this.

This whole technique, if you really want to look at it roughly, is a magnificent column. It is a powerfully effective fractionating technique for taking individual segments of the chromosome and distributing them over a million test tubes. But you can't do that by starting with the total DNA and using a DNA replication system in vitro. You will make more of what you have got, but if you started with a mess you end up with a mess.

DR. SEDAT: Well, say you were to fragment with the restriction enzymes, I mean, just dilute this sufficiently so that it would be equivalent of a single burst experiment, and if you had a sufficiently sensitive replication system, you could well amplify it. I mean, the DNA replication system at Princeton actually will start with something with a fragment of F1, for example, and you will get something ten times the size of before. This represents a very substantial increase because it can be cleaved again with restriction enzymes, which really amplifies at least the DNA.

Agreed, it is a little more difficult, and perhaps a lot more difficult, but it is not inconceivable that this may well be the direction that one could go.

DR. FREDRICKSON: One more minute.

Mr. Madansky, very briefly, please.

MR. MADANSKY: I believe also it is not just a matter of breaking up total DNA and putting it all into a DNA replication system. There are methods for pulling out those DNAs that you are interested in, even with control regions on either side. It is a technique developed by Ray White. If the DNA you are interested in has a messenger RNA or an RNA of some sort, you can put that on the column and then you can get a double-strand piece of DNA to make a triple helix under certain conditions with that RNA.

Now, if fact, that is a way of selecting out specifically or really in reaching for it to a reasonable point, a particular DNA with its control regions as chopped up by restriction enzymes, and then being put into the Bruce Alberts system are reasonable alternatives.

DR. FREDRICKSON: I think we may be moving slightly afield.

Thank you, Dr. Sedat.

I would like to call upon Dr. Goldstein. Dr. Goldstein is from Harvard.

DR. GOLDSTEIN: Would you like me to do the comparison of these two sets of guidelines first?

DR. FREDRICKSON: I think that would be very helpful.

DR. GOLDSTEIN: Okay, I am going to try and do a quick summation, and not go into all the specific details. The details are there, and everybody has copies.

DR. FREDRICKSON: Let us--Dr. Goldstein, excuse me. Let me then amplify for the committee that you are now going to compare the present guidelines with some of the suggestions made by you and others in a document submitted from the Boston DNA Recombinant Group, correct?

DR. GOLDSTEIN: Yes.

DR. FREDRICKSON: Thank you.

DR. GOLDSTEIN: Okay, the first place we differ is the basis for a good bit of this work, and that is, E. coli is opposed to as an organisms of choice.

We still feel that it is the wrong choice, because as everyone knows, it is a human inhabitant. Right now there is nothing better, and we feel if the work is to go on, it should go on in an EK2 EK3 extreme. We also feel that the NIH, through the use of contract support and other types of encouragement should very strongly encourage a search for a better organism.

The second place we differ is--well, I will pick an example of it. There is, in the La Jolla guidelines, a provision where invertebrates, for some reason, are separated from vertebrates and therefore shotgunning experiments are allowed to be carried out. We feel that the dangers inherent in shotgunning invertebrates are just as dangerous as any vertebrate, mainly because of the unknown factor, and that unknown factor is what are all those genes you are shotgunning? They have never been defined, so you are not certain what you are working with.

Okay, the third point is that we believe very strongly that there should be broad-based monitoring committees formed. This is both at the institutional level and at the Federal level, and also I can add something else that Marjorie Shaw suggested yesterday, and that is that some type of informed consent forms for all the workers who are involved in this work should be sent out.

And then, of course, there is the problem of informed consent of the public. But I think that is the role that this committee is playing. I think you are a living informed consent form. You represent the public.

Now, there are loopholes, and I will just pick two of them, because they are technical. There is the issue which Don Brown raised yesterday, and I agree with, which is 99 percent purity. What is 99 percent purity? To a biochemist that is pretty pure. To a geneticist that is another question.

Now, the La Jolla guidelines state that if one can show that 99 percent is obtained with some material, the containment EK, that is the biological, and the P containment, the physical level, will soon be dropped. They can be lowered.

Now, there is something strange about that because no one can define purity. What is 99 percent purity? How can it be confirmed? What are the criteria that are going to be used?

Quite obviously, from the discussion that went on yesterday, that issue is not defined in any way.

Now, I will give you an example. Everyone gets snowed by numbers, 10^9 , that is a lot of zeroes. But if you have one percent contamination, you have 10^{-7} , and that is a lot of zeroes also.

Another loophole in the guidelines that we saw is somehow embryonic tissues were differentiated from other types of vertebrate tissues, and I think that is rather foolish. Embryonic tissues are almost impossible to remove from the follicle cells that they are obtained from.

Okay, now there is--I would like to answer--Dr. Berg asked a very reasonable question, what do I mean by banning. That is a rather harsh term, and I think it deserves an answer. To me it means delaying, and when we suggested experiments should be banned or delayed, what we meant was that they should be delayed until meaningful biological experiments can be done that prove that these experiments are not hazardous. We are not saying they shouldn't be done, but we are saying that there should be a basis for doing them, and some certainty about the hazards involved. There are techniques, I mean, in the whole cancer field. There are basic techniques in carcinogen testing, and the theory is that you give the largest dose for the longest

time. It is called a dose response curve. That is the largest dose which doesn't kill the animal.

We feel those types of experiments should be done. Animals should be given the maximum tolerative dose of E. coli, and then what happens is you look, during the normal lifetime, for instance, and I guess in mice it is two years. You allow that mouse to survive for two years, and then you look to see if there is any sign of transformation, that is, cancer. Those are the kinds of tests that should be done.

Other types of tests have been proposed, that is, to put polyoma, I guess, on a lambda vector into E. coli and then put that into mice. Then the assay would be to look for some rapid signs of virus multiplication or the production of T-antigen.

Well, in some ways those experiments are being set up to give the negative answer, to give the go-ahead to do more experiments. Production of T-antigen is symptomatic of something, but there is no saying that that is what you would be seeing.

Okay, the sixth point--Well, I might also mention in that banning and delaying, there are other points which weren't mentioned yesterday, but I think they are all pointed out to you in the guidelines. The sixth point is that we are opposed to both SV40 and polyoma as vectors, at least until some human epidemiology has been done that would demonstrate that they will not cause cancer.

Then there are other minor points, such as points on physical containment. I honestly do not believe there is any difference between a P1 lab and a P2 lab except that someone puts up a sign. I mean, people walk in and out of those labs all day, and I think P3 containment is the first meaningful level of biological--physical containment. Even that is questionable, and that was raised yesterday.

I can stop there.

DR. FREDRICKSON: Thank you, Dr. Goldstein. Are there other comments or questions?

Dr. Wallace Rowe?

DR. ROWE: In the question of lowered containment for the embryonic tissue, I would like to clarify one point and point out one incident that occurred at La Jolla that I really think ought to be brought up to a general committee on this subject.

We recommend embryonic tissue because of its lack of exogenous viruses. The recommendation at that time did not include germs which I think you were referring to when you said separation from follicles. We are talking primarily about embryos, the fertilized, developing fetus. The reason germ-line cells remain here is because of a comment by Dr. Sidney Brenner, who recommended that they were another area of rather pristine, uninfected DNA, not carrying adventitious viruses. The way this occurred is that interestingly Dr. Brenner tried to justify the use of embryos.

Dr. Brenner raised his hand and said, "Dr. Rowe, where do you place sperm?" Dr. Stetten said, "That is a very personal question."

(Laughter.)

DR. FREDRICKSON: Mr. Hutt?

MR. HUTT: I can't follow up on that.

(Laughter.)

DR. GOLDSTEIN: Can I answer that question, the first question?

I mean, I think you have a point in one area, in the sense of it being pure tissue, but embryonic tissue is still basic in the sense of it being defined in a vacuum, you still cannot define the genes that are there. That is still to me as dangerous as any other aspect of it.

DR. FREDRICKSON: Mr. Hutt?

MR. HUTT: I am unclear about your first point. You first stated that E. coli should no longer be used, and then you concluded your first point by changing that, if I heard you, to saying that EK2 or EK3 should be used. Now, are you--am I simply misinterpreting you?

DR. GOLDSTEIN: No, I will clarify that. I apologize if I wasn't clear.

What I tried to say was that we still feel it is the wrong choice, but there is nothing else available. There is no better choice in the immediate sense.

MR. HUTT: You are then saying that the experiments with E. coli should not be deferred, but you would in a sense recommend a three-step procedure, using EK1 until EK2 is available and then switch to EK2, and all the time be trying to develop something other than any form of E. coli, and then move to that when it is available.

DR. GOLDSTEIN: With a slight modification. I don't think EK1 should be used for all experiments.

MR. HUTT: Well, I understand.

DR. GOLDSTEIN: Other than that, I sort of accept your position. There is an EK2, and I was really pleased to hear that. I hope more work goes into that.

MR. HUTT: But you are not suggesting, in short, that there should be a moratorium until something other than E. coli is available?

DR. GOLDSTEIN: Well, I think some kind of a limit should be put on in the sense that--

MR. HUTT: Could you be more specific? I am having trouble understanding.

DR. GOLDSTEIN: In the original set of guidelines which we sent to the committee before the La Jolla meeting, we suggested two years be given with E. coli.

MR. HUTT: Two years?

DR. FREDRICKSON: Dr. Singer?

DR. SINGER: I want to make a comment about the question of alternatives to E. coli, because I think that at this point it is not clear to me anyway that there will ever be an alternative which in fact offers any advantage, and the reasons are the following.

We know now that E. coli K12 does not colonize normal human bowels. Now, what we said we would look for in an alternative system is something which has a peculiar ecological nature which doesn't include the colonization of humans. The more serious problem is the exchange of genetic information from E. coli K12 to certain other organisms, other kinds of E. coli in other organisms. We know a fair amount about the kinds of organisms which will accept DNA from E. coli K12.

One of the things that we are going to have to learn about any possible alternative organism is whether it can exchange genetic information with a whole series of common and not so common organisms. At this point we don't know anything about alternative organisms in these terms, and it seems to me that if it is not clear before we start that we will in fact find an organism which is unable to exchange readily with a lot of other cloning organisms, and until we know that, then it is not clear that we will ever have an organism that is better than E. coli K12. So while I am certainly not saying that I don't think we ought to be looking for such a thing, I don't think we can go on the assumption that there will one day be something that is better.

DR. FREDRICKSON: Dr. Curtiss?

DR. CURTISS: I won't repeat what Dr. Singer said, but I will amplify it.

In order to have an alternative, that alternative organism has to have cloning vectors. Cloning vectors are plasmids phage, which are ubiquitous among all micro-organisms. Therefore, any alternative, to be useful, will have a genetic exchange system, just like E. coli. So I think that in the absence of a very specific example--I really see no alternative.

I mean, we know more about E. coli than any other organism on the face of this earth. We know something like what one third of its entire genetic information specifies, for example. So obviously I think we know now how to disarm E. coli so that humans are no longer an ecological niche. I think it would be important for any organism that one used to be able to disarm it so that its normal ecological niche would be abolished if it should get out of the laboratory. I mean, I don't see any difference in terms of the requirements that one would demand for some other system in terms of making sure that the inhabitants of its normal ecological niches were not at risk if that organism should get out of the laboratory. I don't care whether the ecological niche is the soil, the moon, the polar ice caps, or whatever. There are organisms that are of importance to the biosphere which inhabit all of these locations, I guess, except for the moon, although they might even be there.

DR. GOLDSTEIN: May I respond for a minute?

DR. FREDRICKSON: Yes, you may.

DR. GOLDSTEIN: I agree with both Maxine and Roy in what they said. I think that putative EK2 strain should be pushed to an EK3 strain. I mean, it may come about that in 2 years or 5 years the scientific community as a whole decides there is no better strain, and that EK3 strain is so foolproof that that is the best strain, but I don't see any emphasis on that. That is what worries me.

I think there should be two pushes. I think there should be a push to get that EK2 strain tested, and a push to EK3, and then have a period of intensive study of that EK3 strain. I am not saying that we have any EK3 strain, but intensive testing and at the same time there ought to be testing of other organisms.

People have suggested B. subtilis. I am not sure. It is a spore-former, and I don't know a lot about spores, but there are problems there. But I mean that kind of work should go on.

DR. FREDRICKSON: Dr. Melnick?

DR. MELNICK: Dr. Goldstein, you asked that epidemiological information be made available before using polyoma and SV40 as vectors. There is epidemiological information. We know that polyoma does not infect human beings. There have been epidemiological studies going on on SV40 now for about 15 years. Inadvertently polio vaccine that contained SV40 was fed to newborn children who have been followed during this period of time. Even though SV40 in large doses may infect human beings, there is no evidence that it produces tumors in them. In fact, there is no evidence that SV40 produces tumors in its natural host, and if the hamster had never been brought into the laboratory as a test animal, we wouldn't know that SV40 was oncogenic at all in any species.

There is no way of transferring the data obtained from hamsters to human beings. Adenoviruses are very oncogenic to hamsters, and there is no evidence that they are oncogenic to man.

DR. GOLDSTEIN: May I respond?

DR. FREDRICKSON: Yes.

DR. GOLDSTEIN: I wouldn't want to challenge your expertise in that area, but I have questions about it. The studies you just mentioned are 15-year studies. What happens 30 years from now? That is one point. We don't know. The question has not been answered.

Then there is the beautiful work presented, to give you an example, by Hanafusa at the tumor virus meetings at Cold Spring Harbor. I think it was with quail viruses, and basically the idea is that one virus may act as a helper for another virus, or it can induce another virus, and if we look for something very direct we say, "Does polyoma cause cancer?" or "Does SV40 cause cancer?" and in a direct sense we may not see that.

But I don't think there is any certainty as to the effect that may have on other viruses, which are in those cells. I think everyone would agree that we are filled with other viruses.

DR. MELNICK: It may take generations.

DR. GOLDSTEIN: That is right.

DR. MELNICK: It may never be provable.

DR. GOLDSTEIN: You are absolutely right, but that question should be raised.

DR. FREDRICKSON: Thank you very much, Dr. Goldstein.

Dr. Shaw?

DR. SHAW: On the embryological question that he raised, I am concerned, too, about the pristine quality of embryonic DNA. I think that we don't know enough about how viruses are integrated into the genome. We certainly do know in man that there are dominant genes that are inherited and are certainly present in the zygote DNA, and we are addressing ourselves only to the question of foreign viruses that infect later. I think we must keep in mind that the native DNA of a number of organisms may have cancer potential.

DR. GOLDSTEIN: It is not just even animal cells. The same analogy exists in E. coli, the organism of choice. There have been papers published, the last one by Ira Herskowitz in virology dealing with cryptic phages. Now, lambda is one of the vectors to be used, and what one would want to do is make a crippled lambda so that one could control it. The best way to do that would probably be through deletion mutants. You actually eliminate a certain gene so it couldn't package or so it couldn't make a tail, or some other important function, essential function.

But what Herskowitz has shown is that lambda genes are present in E. coli, not complete genomes, but lambda genes, and they therefore can rescue any cryptic which is going to be--any crippled vector which is going to be used.

DR. FREDRICKSON: Thank you, Dr. Goldstein. I think we really have to move on.

DR. GOLDSTEIN: Shall I give the second talk?

DR. FREDRICKSON: You have a second talk?

DR. GOLDSTEIN: Yes. It is the talk I was supposed to give yesterday.

DR. FREDRICKSON: How long will it take you, Dr. Goldstein?

DR. GOLDSTEIN: Less than 10 minutes. I will try.

DR. FREDRICKSON: I think I will have to ask you to step down for a moment to see if we have time before we do continue.

DR. GOLDSTEIN: Sure.

DR. FREDRICKSON: Otherwise we certainly want you to provide that to us in writing.

DR. GOLDSTEIN: That was the statement that was given out. I don't know if the committee has it.

DR. FREDRICKSON: We have that statement, then. And you refer now, in that statement, to the document signed by the Boston DNA Recombinant Group?

DR. GOLDSTEIN: This is the one dated 8 February, 1976 that I wrote Sunday night.

SPEAKER: It was handed out yesterday.

DR. FREDRICKSON: It was handed out. We have it.

MR. HUTT: It is on the stationery of the Department of Microbiology of the Harvard Medical School.

DR. FREDRICKSON: Thank you. If we have time, Dr. Goldstein, we will ask you to read that; otherwise we will make sure that it is given the same careful consideration that it would have received had you spoken it. Thank you.

DR. WIESENFELD: I would like to respond to what Dr. Melnick said. Can I make just one short comment?

DR. FREDRICKSON: Yes, one short comment.

DR. WIESENFELD: I would just like to say that a virus--

DR. FREDRICKSON: Would you identify yourself?

DR. WIESENFELD: I am Dr. Wiesenfeld.

DR. FREDRICKSON: And would you stand up so that you can be caught in the mikes?

DR. WIESENFELD: I would just like to mention that a virus indistinguishable from SV40 has been isolated from a progressive disease of the central nervous system, progressive multifocal leukoencephalopathy. I think references to that are in the guidelines in regard to SV40. So we don't really have total evidence that SV40 is benign over a long period of time for humans.

DR. FREDRICKSON: Thank you.

Dr. Rowe? You have asked to speak as a member of the committee. Dr. Rowe, I shall be tough with you on time as well. Can you finish within ten minutes?

DR. ROWE: Oh, certainly.

DR. FREDRICKSON: Very good.

DR. ROWE: I even promise to behave myself.

(Laughter.)

I wanted to try to get a little different perspective to some of the risk problems that I see. First, I think one very important thing to recognize is that these guidelines are hopefully very temporary structures. Asilomar was a step, these guidelines are a start up a little bit, and mostly based on the fact that we really don't know much about what the risks are and what might happen.

But I would like to get across one feeling I had yesterday, which was that very little attention was paid to the reality of these risks. They were always referred to as theoretical, potential, and I don't think they are really that distant. I think the important logical step to put across is that one can very readily envision deliberately creating the most horrendous micro-organisms by means of this technology. I am sure that the BW workers are hard to work putting everything into--or at least making scenarios where they would put all kinds of harmful toxogenic and antibiotic resistant genes into E. coli. One could do it deliberately, therefore the possibilities exist that one could do it accidentally. I think those are real.

One minor scenario is that everyone agrees that it would be totally irresponsible to put botulinus toxin gene into E. coli. One can move back from that by degrees. It would be equally negligent to put Clostridium botulinus shotgunned DNA into E. coli. It would be equally--well, one point of background. Botulinus toxin is not basically a bacterial function. It is equally a phage, a virus function. The virus carries the genes for this toxin from one botulinus strain to another, and makes it a toxinogenic strain. Therefore you should never take a gene pulled from botulinus phages and put it into E. coli. You would wind up with the same results.

Conversely, or additionally, you should never take phages that you don't really know much about and put their genes into E. coli, because you might well have an analogous system. It is not just botulinus, it is diphtheria toxin, streptococci, and I believe other clostridial toxins that are under phage control.

Putting phage genes into E. coli could well create a toxinogenic E. coli that is not anaerobic, that doesn't need just badly canned beans in order to get to us. It could be all over. I think these risks are very real. I think they are very minor. Of all the possible DNAs that can go into E. coli in the course of experiments, a tiny, tiny fraction will actually be dangerous. We could name a few, and I think that is important, and we can't name those that are absolutely not dangerous.

The reason the guidelines are so conservative is because we don't know, but secondly, if we didn't know and we did not have a chance to know whether these are going to be dangerous experiments, then we would have to commit ourselves to either being conservative forever or taking our chances or taking other people's chances forever. But we are in a system where we can evaluate the risks. We are in a very poor situation now for evaluating risks, but it is feasible to do the experiments to find out the risks.

So that is one reason why I think we should stay very conservative, because there is a chance of making more informed decisions when we do the right experiments.

Now, the committee, the DNA program advisory committee has appointed a subcommittee under Dr. Szybalski, to look into the question of what experiments can be done to evaluate risks better, and I see that as the major function of the committee from here on out, to do good experiments and get the input from the whole scientific community as to what experiments are worthwhile, interpretable, convincing, and how to do this very important job.

As another job of the committee, Malcolm Martin and I are doing this experiment that has been alluded to on several occasions of seeing if polyoma DNA inserted into a cloning vector can get out and initiate infection in an animal.

In this regard -- how is my time doing?

DR. FREDRICKSON: About three more minutes.

DR. ROWE: In this regard I would like to point out a very important way of analyzing things that has not really been alluded to except once by Paul Berg. How might these experiments be dangerous? The Asilomar listed the three mechanisms, and I think they are always important to keep in mind. There are three general categories whereby recombinant DNA experiments--We are only talking about E. coli. We are not talking about tissue culture cells, or normal viruses. Just normal biological systems. How might these be dangerous experiments?

They might disturb regulation, that is, change the behavior of the E. coli so it occupies a different level of the gut, so it penetrates more between villus cells, so it gets into the lymphatic system, and all kinds of minor changes in E. coli could change its balance within the organism.

It could introduce -- it could serve as a vehicle for conveying high concentrations of nucleic acids into or next to cells. The danger of getting viral genomes into villus cells or whatever, macrophages, viral genomes transforming cellular genes could be brought into proximity to

intestinal or other cells and could create transformation, transduction types of insertion of new genetic information into our cells.

Thirdly, they could produce a disease by means of products, the enzymes, the cellulase, the example that was given yesterday, botulinus toxin, insulin, and an enzyme that changes a pathway in E. coli and makes a metabolite into a toxic metabolite.

So this can impinge here, but these can cover almost all of the cases that one can think of. Now, there is a very crucial variable that I think ought to be brought into the guidelines soon, when we understand it better, and it is also crucial for evaluating risk. That is, as Paul Berg alluded to yesterday, a question of whether the E. coli translates, and it is an insufficiently discussed variable.

If E. coli translates the foreign DNA it can be a danger because of the regulation problem, particularly because it can make new pathways. It is irrelevant to the question of transferring nucleic acids, except it might facilitate the transfer, and of course the products are the big danger in a translating E. coli system.

However, if it does not translate, the products automatically are no worry; the transfer of the nucleic acids is. And the question of regulation is minimized because it only changes regulation by inserting into a controlled area where the RNA is competing for ribosomal sites or some such, many fewer scenarios for producing bad results.

Now, can one evaluate these hazards? I think the experiment of the polyoma in E. coli or in lambda can evaluate this mechanism very efficiently. If the experiment is done with enough variables repeated enough times, it covers a lot of ground. In response to Dr. Goldstein, we will not tip this experiment in favor of negative results. We are not going to stop if we get a negative. We are going to keep on going until we are convinced that we won't get anything except a negative. We are leaning it toward getting positive results.

So I think we can evaluate this risk scenario and very possibly eliminate it as a serious danger. I think this one also can be evaluated experimentally. I think if one takes shotgun clones from Drosophila or frog, or whatever are considered the best to work with, and characterizes 100 or 1,000 of them for, Did they change the behavior or the pathogenicity of the K12 that they are in? One can at some point say okay, this is a rather ridiculous mechanism; we don't have to worry about it.

So I think this can be made a set that we don't have to worry about. I don't think we can evaluate these, because each is a case of its own. But this is a tremendous step, because a nontranslating system is useful for two of the three major uses of this technology. You can clone with it

and you can produce DNA with it and you can produce RNA with it as reagents. You just can't produce proteins. But this should be the goal, and I think we may well find, or my hunch is that naturally occurring E. coli are all in this box, and that if you work with the nontranslating systems you don't have to worry about it becoming a translating system in nature.

I think you are going to have to work very, very hard to get a translator if one exists in nature, so I am saying I think we can make informed judgments, but it is going to take time and hopefully we can get rid of the more ridiculous parts of the guidelines. But they will only be ridiculous in retrospect. Right now they are very, very necessary.

DR. FREDRICKSON: Thank you. Dr. Rowe is a member of the committee which has prepared these guidelines.

Now I would like to move now to call Dr. Marshall Edgell. Oh, I am sorry, Dr. Sinsheimer?

DR. SINSHEIMER: I don't want to ask a question, but I know there is some evidence with regard to translation that I think should be brought out right now.

DR. FREDRICKSON: Would you please do so?

DR. SINSHEIMER: Dr. Berg and Dr. Hogness are the people who have the data.

DR. FREDRICKSON: All right. Dr. Hogness?

DR. HOGNESS: I mentioned some of it yesterday, on one side of the fence, and I think what Dr. Sinsheimer is talking about is on the other side.

Yesterday I mentioned that we have evidence that the frequency curve unit length of DNA in Drosophila and yeast of translation is small. It is reduced by about two orders of magnitude, 100-fold at least, compared to bacteria.

On the other hand, there is some evidence from Ron Davis's laboratory that the transformation, or a function for histidine, for a particular enzymatic function in E. coli, can be donated by yeast, again with respect to the frequency he doesn't know in that case.

So what it looks like at the present moment, and I think we need a lot more data, is that the probability of translation per unit length of DNA that you are inserting from the eukaryote is much smaller, but that it may exist is still quite probable, once in a while, at low frequencies. Is that a fair summary?

DR. FREDRICKSON: Dr. Berg, do you wish to add to that?

DR. BERG: I think it is fair to say, in spite of what Dave said, that there is not yet any concrete evidence of accurate expression of any eukaryote gene in a prokaryote. There is evidence of new products, polypeptides, but the origin of those and how they are produced is not clear.

What Ron Davis has found, in a paper which has just been submitted, is that it is possible that a DNA segment from a yeast can provide some functions which allow an E. coli cell requiring histidine to grow in the absence of histidine. How it does it is quite obscure. So at the moment I think it is not clear that there is accurate expression of the genetic element in an E. coli system in the way it is expressed in the host of origin.

I think I tried to indicate that yesterday, and I think it is a terribly important point in evaluation of this. But it also has another component, which if I can take another minute--

DR. FREDRICKSON: By all means.

DR. BERG: If we expect to benefit from this methodology in the sense in which I indicated yesterday, that is, to take genes from organisms, complex organisms and introduce them into simple organisms and hope to express them in that host, if expression is rare, we can assume it is possible, but very rare, then we may of course not get the benefits that we are projecting.

On the other hand, it is in E. coli, and very likely only in E. coli, at least for the very foreseeable future, that we know enough about its genetic systems to hope to manipulate that inserted segment in such a way that it becomes translatable. That is, it is possible to imagine taking a segment out of animal cell, introducing it into an E. coli, but not putting the appropriate signals, again by manipulating signals in front of it and in back of it to make it indistinguishable from a prokaryote gene, and ask the E. coli now to express it and translate it as if it was one of its own. The hope of doing that with any new organism, the messiah of the future, I think is just so difficult to contemplate, I think the benefits are likely to come from our understanding of knowing E. coli. The ability to protect ourselves also comes from our familiarity with E. coli. To me, it seems that E. coli is by far the organism of choice rather than going to look for some completely unknown organism which we will never--well, not never, but certainly only in the very distant future, hope to be able to manipulate to that extent.

DR. FREDRICKSON: Thank you, Dr. Berg.

DR. WRIGHT: May I make a comment, please?

DR. FREDRICKSON: Very briefly, please. Would you identify yourself?

DR. WRIGHT: Susan Wright, from the University of Michigan.

I think what Dr. Rowe has said has clarified for us all, that regardless of testing what the risks might be. What I am concerned about is the question of the timing of these experiments. Are they going to be done before the recombinant DNA experiments? Ideally I think that would be the best solution.

Also, I think they should be done in a few restricted labs, at P4 before any other experiments are done and before we allow a proliferation. I think the question of timing, when these tests will be done, is exceedingly important.

DR. FREDRICKSON: Thank you, Dr. Wright.

We will have to move now, taking my prerogative to keep this on tract, slightly. I will call upon Dr. Marshall Edgell; and then, Dr. Goldstein, would you be preparing during Dr. Edgell's presentation a five-minute summary of your second point? I do want you to be heard, but we are going to have to come then, without discussion, to completion of the public testimony within the next few minutes.

Dr. Edgell is from the University of North Carolina.

DR. EDGEELL: Well, I think I can be brief. I am in the Biology Department and Curriculum of Genetics at the University of North Carolina. I am interested in gene regulation and applied genetic engineering and have been doing recombinant experiments with sea urchin DNA for several years now. In my opinion, the proposed regulations are too conservative.

I would like to alter the statement that I previously submitted to the committee, since many of those points have been elegantly presented already. I would like instead to concentrate on the major philosophical point which has been troubling me.

These recommendations embody, in my belief, an approach to intellectual activity which represents an unusual departure from the precedents of free inquiry. This departure is not in that we are attempting to evaluate the risk of a scientific activity in order to protect mankind, nor that we are about to impose regulations on scientific experimentation. To bring social pressure to bear on professional behavior is laudable indeed.

What seems unusual in this case is that we seem to be requiring excessive precautions considering our previous experiences with genetic materials. Apparently it is the fear that these experiences inadequately represent the new circumstances which is motivating these recommendations. We seem to

have reached a consensus concerning our lack of ability to predict with certainty the outcome of these experiments. What are we to do in the face of such an awesome dilemma?

Why, proceed cautiously, of course. But what in fact constitutes truly cautious behavior? Would it have been prudent to withhold the polio vaccine for, say, ten years, to appraise all of the unknowns? It is possible to be too careful, just as it is possible to not be careful enough. So what are we to do in the face of our uncertainties? We have dealt with such issues over and over again in the past without gruesome consequences. In the past we have acted on the basis of our admittedly inadequate information which was at hand.

All of my experiences and the evidence I have heard cited suggest that these experiments are unlikely to be catastrophically hazardous. The experience of those who work with pathogens tells us that we can be trained to work with hazardous agents should they arise with no unreasonable risk to workers of the population at large.

The argument that some unknown factor may arise cannot itself be contested. It must be recognized that this same logic can be used to block virtually any area of human activity. It is a paralyzing position. Unless we feel that the societal contributions of science have come to an end, it seems dangerous to establish the precedent that we limit our explorations to areas in which we know for certain what will happen.

We can be confident that this area of research will be as difficult as any other and hence will require major contributions from numerous scientists. To get these contributions will require a vigorous and health pursuit of this area of knowledge. Science cannot flourish in an environment hemmed in by an endless flow of paperwork, loyalty oaths, and the need to justify and obtain permission for every modification of the scientific protocol. This burden on creative activity is of course justified if there is a real hazard, but it exacts a heavy price for naught if the risk is one only of the imagination. It seems ironic, indeed, that for many years basic biology or genetics was criticized for not having made any contributions to the lives of individuals and now that we are on the verge of doing so that age-old specter of things-that-go-bump-in-the-night arises to cloud our efforts.

It is clear that there will be rules of some sort controlling our activities in this area of research. If the scientific community feels that these experiments represent such an unusual case that we need to abandon the precepts of using existing knowledge, I feel two conditions should be added.

I recommend that along with these recommendations go funded contracts to demonstrate the purported hazards of these activities. The uniqueness

of this current situation would be acknowledged by the second condition that if no hazard can be demonstrated within, say, five years, the regulations should be automatically terminated. In that case we would return to the normal circumstances dependent on investigator integrity and personal responsibility for one's actions. I am aware that at least one project has been started to explore the hazards of this type of experiment.

What I am recommending is that we set a time limit and if no hazard can be demonstrated in that time, we automatically terminate this unusual set of rules concerning experimental activity. If the hazards are as severe as some people suggest, it would be easy to demonstrate.

Thank you.

DR. FREDRICKSON: Thank you, Dr. Edgell. Dr. Edgell's revised statement is now being Xeroxed, and will be distributed to the members of the committee.

Dr. Goldstein? May I call upon you for five minutes further?

DR. GOLDSTEIN: This is from the February 8th document.

DR. FREDRICKSON: Dr. Goldstein refers to the statement dated February 8th.

DR. GOLDSTEIN: What I will do to try to get it into five minutes is to just summarize the first half of it, and you all can read it, I hope. Then, the last part of it is a statement from the critique we wrote, and I would like to read that.

In the first part of it I tried to summarize a lot of the questions, and I think that will be most useful to people in the audience just to look at them to refresh themselves. Then there were two issues that I brought up.

Well, let me read something from the original--I guess the Wood's Hole guidelines, where they are referring to biological and physical containment barriers. The statement is, "The level of containment provided by these barriers should match the estimated potential hazard for each of the different classes of recombinants. For projects in a given class this level should be highest at initiation, and modified subsequently only if there is a substantial change in the assessed risk or in the applied methodology." That is what we are hoping will be applied. That is from the Wood's Hole statement.

Now in relation to that I think this committee should realize you are going to be setting two important precedents that were mentioned briefly this morning. The first is that your conclusions are going to be a signal as to the types of safeguards which will be taken by similar bodies in other countries throughout the world. Sidney Brenner made this point very

elegantly. You know, we see lots of numbers, again, and they are large numbers, and they are impressive, but when you increase the number of labs exponentially it should be obvious the effect that has on the probability of accidents. Likewise, the same thing would occur with industry. Industry is interested in this. ICI in England and General Electric, as I stated in this statement, have either shown strong interest in doing the work or are already involved in doing it.

Now, I would like to read from our original critique. "Many biologists have worked hard and long to draft the Asilomar statement and Wood's Hole guidelines [and also the La Jolla guidelines]. We admire this work tremendously and feel it is a very positive development when a community of researchers join together planning to eliminate hazards from experimental design. The initial unprecedented response by scientists to a new technology, calling a moratorium and the subsequent Asilomar meeting, and saying there are certain things that we are wise enough to refrain from, brought public approval on a wide scale.

"Now, the situation in practice and on paper is in flux. Our own current position is that recombinant DNA experimentation is valuable in selected instances and hazardous in others. The problems facing the guidelines committee is to choose carefully those few experiments which will lead to societal benefit from a collection of innumerable, possible experiments, many of which will be too hazardous to merit being done.

"In stating that an experiment creating a new organism is too hazardous to be done, it bears clear emphasis that there is no issue of freedom of inquiry. We fully support freedom of scientific inquiry. However there is here an issue of freedom of manufacture - the manufacture, in this case, of self-replicating, novel organisms, which are potential global health hazards or ecological hazards....

"The difficulties of estimating by theoretical considerations the potential hazards of DNA recombinant experiments appears insurmountable. A comparison might be made to the dangers involved in using radioisotopes in biological research. With radioisotopes the direct dangers to lab personnel, dangers of disposal into the water supply or into landfill, etc., can be quantified. It is known that exposures to certain levels of radiation is dangerous, whereas exposure to certain lower levels is not. It is thus possible to say this level of safety (procedure) is adequate while that level of safety is inadequate.

"The situation is fundamentally different in the case of recombinant DNA experiments. Here the dangers can not be quantified. They can only be guessed at over an extremely broad range of probabilities (from high to low) for any single given experiment. It is thus very difficult, and indeed, somewhat unrealistic to say this level of safety (procedure) is adequate,

while this level is inadequate, as the Asilomar and Wood's Hole [and now the La Jolla guidelines] have attempted.

"Rather, an inevitable, prudent, philosophical approach for this type of hazard seems to be one which says we will do those recombinant DNA experiments judged to be worthwhile in as safe a way as possible. This approach is justified because the potential hazardous consequences of the DNA recombinant work are extremely serious, and because of the all-important distinction between quantifiable, (e.g., radioisotopes) and nonquantifiable, (e.g., recombinant DNA) hazard probabilities." Only strong and tight guidelines will allow this approach. The social benefits which may arise from DNA recombinant work will be of equal value whether they come in 20 years versus 25 years; 50 years versus 55 years; 100 years versus 105 years. They will endure, if valuable, for many centuries thereafter. For five or ten years now, a slow, thoughtful, research-based approach to limiting hazards makes sense.

DR. FREDRICKSON: Thank you very much, Dr. Goldstein. I certainly do appreciate your reading of this statement.

I would like now to call upon Dr. Stetten, who has been allotted five minutes.

(Laughter.)

Dr. STETTEN: I will sit here in order to avoid wasting time. I went over my notes last night to fill the assignment of summarizing. I have listened carefully this morning. Summarizing adequately is obviously impossible. The present confusion clearly, I think, arises from the fact that we are being asked to offset hypothetical hazards with speculative benefits. It is a familiar problem to me, because I saw it in the first meeting of our advisory committee, that there were widely divergent viewpoints, because, really, of the absence of hard data.

I would just summarize, if I may, some of the divergences which we have heard in the last day and a half. On the one hand, Dr. Fredrickson, you have been advised that the guidelines that were drafted were too permissive. On the other hand, you have been advised that they are too restrictive.

On the one hand, you have been advised that we have moved too slowly. On the other hand, you have been advised that we have moved too rapidly.

On the one hand, you have been advised that the language of the guidelines is too strong in that it embodies law, rather than guidelines. On the other hand, you have been advised that it is too weak, that it uses the conditional mood "should," where it should have been "shall."

On the one hand, you have been advised quite recently that E. coli is the most desirable organism for this kind of study. On the other hand, you have been advised that it is certainly not the most desirable, and perhaps far from the most desirable organism.

In trying to provide you with a starting point, I would like to mention that up the coast from here, if one sails, one soon encounters the Island of Newfoundland, which is separated from the mainland of Canada by a very narrow strait, the Strait of Belle Isle. The Strait of Belle Isle is a fog-ridden part of the water, yet the sailors up there sail it in dense fog with impunity--I have read.

(Laughter.)

By listening to the surf on the left, and listening to the surf on the right, and when the surf on the left and the surf on the right are precisely equal, they know that they are charting a safe course. This analogy is, sir, the best advice I can give you right now.

(Laughter.)

(Applause.)

DR. FREDRICKSON: That does explain the roaring in my ears.

(Laughter.)

The chair would like now to take about 5 minutes himself, to indicate where it is we go from here, both in this meeting and following its adjournment.

In my opening remarks I noted that recombinant DNA experiments do bring to the fore certain problems in the public scrutiny of the process of basic science, and one need not repeat the suggestion that this field is indeed extraordinarily complex, but also that it appears to be vital that there be public awareness and understanding of the process.

Now, yesterday's meeting, and I think this morning's thus far, have been very helpful. They have served to provide an airing of the issues. I think that all sides have been presented, and there has been opportunity for comment from both committee members and the public, and there has been, note, an opportunity for questioning of those who have spoken.

We have thus created a public record of a hearing that supplements the proceedings of the NIH committee, and one that should assist in the process of establishing guidelines for this research.

Now it is time for the committee members to respond to my earliest suggestion--that is, that I would like to gain from them in brief terms a sense of their comprehension of the nature of the experiments and risk-benefit assessments, and what judgments, if any, they have arrived at after hearing all sides of the issue as presented. Your response to the proposed procedural mechanisms for implementation and monitoring the guidelines are also important. We have had less discussion of this than we have had of other issues.

Now, before asking for your comments, I would like to review some possible steps in how we will proceed from this meeting. I would like to remind all of you that there will be no vote taken. This committee is advisory to the Director, and it is from the comments of the committee as well as the entire record that that decision must be made.

Let me, in very non-legal terms, explain briefly the nature of guidelines and what may follow from them.

Mr. Riseberg of the General Counsel's Office is here to amplify or answer further questions, and I know that several members of the committee, particularly Mr. Hutt and perhaps Judge Bazelon, will in their own comments clarify much of what I am about to say. But basically we are here today to discuss guidelines which are to determine how certain research which is funded by the National Institutes of Health should be carried out--the basis of which we would expect to award grants and to continue support for such research.

Guidelines in general have little legal standing. Their enforcement is really up to the institution and to the NIH, and they usually are followed by a manual issuance. That is, when the guidelines have been agreed upon, a manual is issued indicating what the guidelines are. These, then, also have limited legal standing in the usual case.

What, then, can follow the issuance of a manual? Well, there are two possibilities. One is that we can then put the guidelines referenced in the manual as a notice for the Federal Register. The guidelines here are proffered as a draft working document. This is an optional first step in developing regulations, and it allows the public opportunity to comment before guidelines might be proffered as a notice of proposed rulemaking.

The notice of proposed rulemaking is a step which can be taken after the manual issuance, and it can bypass the notice for the Federal Register, which is a more leisurely period for the development of further comment and experience with reference to the guidelines that have initially been approved.

Now, the notice of proposed rulemaking has definite legal standing, and again, it offers an opportunity for comments by the public before those

rules would become regulations. The last step then would be the development of regulations, the legal standing of which has been definitively established, although even here I think actions in the courts can affect the standing of regulations in regard to constitutional and statutory and other common law issues which they may cut across.

Now, what do we propose to do from here? First, I would like to emphasize that no decision will be made today, that we will make the final decision on the basis of the record. It would be to make a charade of these proceedings were we to have intended to move otherwise, and it is therefore urged upon all of those who are here, committee and public, to remember that although they may not have said all they would like to have said at these hearings, they are invited to expand upon those comments to us, to me, in writing, for indeed it is to establish as full a record as we can possibly do that is in the best interest of all of us who are concerned with this problem. So you will have ample opportunity to respond to what you have heard today.

I shall expect to have further consultation with the committee who developed these guidelines, and possibly with other experts. It is my hope, then, that this process can go on with all deliberate speed, and I note that this committee developing the guidelines meets again here in Bethesda on April 1 and 2, and I should hope at that time that we can have definite considerations available for it if there are to be modifications that we would ask it to consider for adoption.

Now, this then is a brief summation of the path from this point. I would recommend that before we pick up the extension of what I have said, that we pause for a very brief coffee break, and that within 10 minutes, and certainly at the most 15, we return here to begin hearing from members of the committee with reference to their comments.

(Brief recess.)

DR. FREDRICKSON: I would like to clarify two of my remarks, because some confusion has resulted from them.

First, in requesting that members of the committee and others here today take advantage, if they wish, of the opportunity to send expansion of their comments, I would like you to do so within the next 2 weeks. In fact, for the committee I am strongly requesting that you do send either a reiteration or an expansion of your comments to me. I definitely would like to have those within the next 2 weeks, and that goes for any others who might wish to respond to that invitation.

The second point of confusion is that I referred in April 1 to a meeting of the NIH committee. Clearly we are consumed with committees. I did not refer to this advisory committee, which will not meet again necessarily

on this subject. I referred to the NIH Recombinant DNA Molecule Program Advisory Committee, or more simply NIH Recombinant Advisory Committee, which has developed the guidelines. They will, because they are a continuing group, meet again April 1.

I would emphasize again my realization of the pressures for the solution to this problem, and finally, I have been reminded that it may be of interest to some of you who may not know, what the NIH has experience in enforcing guidelines or making them work with regard to human experimentation. Here, there have been mechanisms developed through which the institutions conducting these clinical investigative activities work with the NIH in enforcing and continuing the development of the guidelines under which they proceed. So this is not an entirely new or alien area of the NIH in regard to another form of experimentation.

Now, if the committee will not take umbrage at what I have to say, we have enough people so that a mean time of about 5 minutes might be desirable for your comments, at least for the first round. I would remind you again since we will be very eager to have you send up perhaps your fuller comments by mail, that you may hit only the high points if you so desire here. Or, indeed, you may pass if you like.

I am going to start then from my left, if I might call first upon Judge Bazelon.

JUDGE BAZELON: Thank you.

Socrates once said it is a wise man who knows what he doesn't know. If Socrates was right, I am a very wise man.

Of course, I wouldn't dare to express an opinion; I have none on the question of whether or not the guidelines are right, wrong, or in the middle. That goes to the merits of the question, and as far as the procedural aspects of it are concerned, I am under even a greater disability, because these are questions that may come before the court, and I must be very careful about that.

But I do think I can say one thing, and that is that whatever decision is made, Dr. Fredrickson, I think you can't go wrong, and you are probably very right, and you would be very safe, in making sure that the decision that you render has within it great specificity for the reasons why you came to the conclusion that you did. Not only am I talking about specificity, but I mean to refer to what was said over these three minutes, the comments that were written and orally presented, what you accepted and what you rejected, why you accepted it and why you rejected it.

Now, I have said too much already, but I would urge that because I think first of all, quite apart from the law, and quite apart from any

cases, the fact of the matter is that the public is entitled to know this. And this problem that we have here today, which Dr. Stetten just pointed out, is that we don't know. We have got a lot of them. We have got a barrel full of them. They are all over the place, and the healthiest thing that can happen is to let it all hang out, works and all, because if the public doesn't accept it, it just isn't worth a good damn. That is what you have to reach.

Now, I don't think that compromises expertise at all. The expert must understand that there are other values in the community. These are the values that have to be balanced, and there is not going to be a satisfactory answer to the problems that are presented here. There can't be. So it is going to take some guts, but you had better back it up with a very good record. God bless you.

(Laughter.)

DR. FREDRICKSON: Thank you very much, Judge Bazelon. I realize the restriction under which you do work as a member of this committee, but your presence has been an extraordinary help.

Dr. Sinsheimer?

DR. SINSHEIMER: Okay. Socrates is a hard act to follow.

I just want to make three points rather quickly. The first is that I would like to point out that I think the research we are talking about here at this meeting really marks the advent of a whole new era, the real turning of the corner in biological research. It marks the transition of biological research from a primarily analytic phase to a much more synthetic phase. And I don't think the full implications of that have sunk into any of us, and it may well be that this will require changes in the modes in which we do research, in the agents that we use, and so forth.

Secondly, I would like to say that I think some of these issues, such as, Is E. coli the best host or not the best host? have been approached in too global a manner. Some of the postulated benefits probably can only be obtained in microorganisms, and for some of those the risk is not great, and for those maybe E. coli is the best host. Some of the other benefits I think very likely could be obtained in other ways with other vectors, and in some of those, in view of the risks, other possibilities might well be much more desirable. I think the problem has to be fragmented and looked into in more detail.

The third point I would make is one that has been implicit, I think, in what everyone has said, but I haven't heard it said expressly, and that is that what we are doing, I believe, is almost certainly irreversible, that

knowing human frailty these vectors will escape, they will get into the environment, and there is no way to recapture them.

The hazard, if there is a hazard, will not be like DDT or PCB or aerosol propellants. In those instances, if one finds there is a hazard, you stop the manufacture, and in time at least the hazard will disappear. In this case, obviously, that does not apply. I think that has to weight the decisions that are taken. I think you have a hard job.

DR. FREDRICKSON: Dr. Shaw?

DR. SHAW: Dr. Fredrickson, I think I can summarize my position with two statements, one substantive and one procedural.

DR. FREDRICKSON: Dr. Shaw, I believe you can be recorded, but please speak up a little.

DR. SHAW: All right.

Substantively, I don't think that our advisory committee can come close in a day and a half or even with a 3-week course to come up to a level of understanding of the guidelines that have been promulgated by the NIH advisory committee. I would prefer to leave that issue in the hands of those that have worked so diligently over the last year and a half, and have come up with four separate working papers.

To continue, that the guidelines as they were drawn up at Asilomar were in effect, that the La Jolla guidelines become in effect, a continuation in flexibility, and change of the guidelines, continue with the input of yesterday's and today's statements being carefully weighed by that committee to consider the substantive issues of experiments to evaluate the risks, to search for alternate organisms, to consider extending, or expanding or contracting their no-no list of experiments.

I am primarily concerned, and I think this committee could address better the procedural problems of monitoring and enforcement and surveillance, and I am very concerned about this. There is very little stated in the guidelines as to how this would be done.

One of the statements on the agency being able to review any requested changes of a principal investigator unless the agency felt it needed outside expertise to make a decision, I think this should be carefully reconsidered and should go back to peer review if there are substantive changes in the direction of the research.

I am concerned about the principal investigator's statement of responsibility. The public biohazards statement, I think, should be made public.

It should be signed. It should be available. All the names of the investigators should be known to the public, and that the principal investigator and the institution share responsibility for the eventuality of damage being done.

I am concerned about informed consent, particularly for all of the people that are associated with the laboratories, the secretaries that enter the labs, the janitors, et cetera. I don't mean to be reversibly chauvinistic, but I think that there is some argument to be made for male technicians in this work rather than female, because of the danger of E. coli infections in the urinary tract during pregnancy, and I think that these sorts of things should be considered. Certainly any woman in the reproductive age should be warned of hazards to a fetus as well as to herself.

I think the local biohazard safety committees, the institutional review committees, and the peer review committees should also be carefully reviewed in terms of what their charges are and how they would best monitor and survey the ongoing experiments.

DR. FREDRICKSON: Thank you.

Dr. Handler?

DR. HANDLER: Thank you.

I have been impressed with the historic character of what you are doing rather than precisely what is being done. I think Dr. Shaw has just stated a long set of caveats which require much more discussion that you will get around this table today or in the next several weeks, and will require very careful thinking through. And that means that this process is by no means done. We have a long way to go in it.

That some problem arising within the context of scientific endeavor should have been examined this way before the government itself decides what position it will take is really historic, and you are very much to be congratulated for getting as far as you have done, and I share with Judge Bazelon the concern with where you go next and I don't envy you that task.

I found it very troublesome that I could sit here for a day and a half and seem to agree with everything that was said.

(Laughter.)

Usually at least I can dismiss some part of what I have heard, but that wasn't so. I found myself agreeing with everyone in turn in a very real and sympathetic way, and that meant that I didn't know my own mind, it certainly indicated to me that the confusion was worse than I thought it was as I arrived. Dr. Stetten described it as being a contest between, I think he

called them speculative benefits and imagined hazards, and that does seem to be the circumstance.

I was sitting, appalled, realizing how far it is to go before you can really have a prokaryote happily manufacturing insulin for you, which is one of the imagined or speculative benefits. It certainly means that there is a great burden upon the NIH, it seems to me, rather than on the great scientific community to do it, to engage in a set of orderly studies which it either carries out itself or sees to it that they are conducted somewhere. That certainly is true with all the elements of safety. The studies that Dr. Rowe brought up this morning certainly were right on target, and those require doing. They can't be farmed out to the academic community, or think that they will spontaneously occur there. You have the facilities and the money and the people. The burden of dealing with the safety problems I think now lodges here very firmly.

That, when you are done with it, will allow you to play games with the flexible guidelines, and I hope they remain flexible for some time.

I guess I was personally troubled by the fact that the nay-sayers were so very young as compared with the aye-sayers. That must be telling us something, but I am not quite sure I know just what it is. In the past, the previous history, conservatism has been the role of elders of the tribe, and the last day and a half, it has been the other way around. That is rather remarkable, and very deeply troubling.

In any case, I guess I do believe with respect to the guidelines themselves that they are a thoughtful document, and I am sure that they will change in due course, and that each piece of them will have to be taken on their own merits; and that is precisely what Bob Sinsheimer was saying a moment ago. I don't think one can hear debate whether any strain of E. coli is the best strain to be using in perpetuity; it certainly is not.

I share all the concern with respect to what physical containment actually means. Things escape from physical containment no matter how tight the containment. I would agree that I don't understand what the P1 containment really is, or the difference between it and the P2. If you are serious you will go to P3. I don't think P1 and P2 contain anything at all, honestly.

The chief difficulty in all such work is that there is not instant feedback for your own blunders, and if only one could arrange that somehow, then the problems which have long characterized work in the clinical microbiology laboratory would be much more easily solved. By and large, those of us who work with toxic chemicals got almost instant feedback. Either you keeled over or it stank, and you knew. In this field you just don't know. It has been the same problem with radiation. We have watched

every laboratory that has worked with radiation become sloppy in the course. Not until they have an accident and something calls them up does that trend reverse. That will happen in this field as well. Meanwhile, the scientific benefits of this work are something I do understand and appreciate, and I think that you have no choice but to see to it that that scientific work does indeed go forward, but the pace at which it shall go forward may be up for grabs for the moment.

DR. FREDRICKSON: Thank you, Dr. Handler.

Dr. Sprague?

DR. SPRAGUE: I will omit certain things that have already been said. I am sure as we go around the table everything will be said probably more than once, so I will try to restrict my comments to the things that have not been dealt with, although I would like to emphasize one or two points.

First, I would like to applaud you and the NIH for the forthright manner in which you have developed a process for trying to arrive at the most logical and defensible position, and both best for science and for the public at large.

Also, I have been very impressed with the representatives of the scientific community over the last 2 days, and what I read before I came here regarding the thoroughness and the diligence with which they pursued this, and their concern for the public good, I might say.

I think that although this has been stated, I would like to reiterate the need to define as precisely as we can, through probably contract research, the risks at their earliest possible moment. I have only an impression regarding the guidelines per se. I would not--I think we are in no position to say, but I would only say that I would hope that you err on the conservative side in the final decision.

I have heard the statement made repeatedly, and almost glibly on occasion, that the investigators throughout the world would adopt whatever guidelines are established. I wish I felt so certain of that. In fact, the greatest risk to me is that they will not, in fact, be followed throughout the world. And I would hope that the advisory committee would address this problem as to how they would ensure, both in this country and abroad, that whatever guidelines are ultimately established will be followed. That is all.

DR. FREDRICKSON: Dr. Koshland?

DR. KOSHLAND: Well, it is certainly easy to make decisions if all the data are in, and obviously, all the data aren't in on this question. However, there is an unevenness of available data, as Dr. Rowe said so

clearly this morning. There is good evidence that prokaryote gene segments will replicate and translate in other prokaryotes of other species. I think probably that with this information the rules that have--the guidelines that have been written are pretty realistic and will suffice as temporary rules, so to speak, for conducting research in this area.

On the other hand, of course, there is the question of whether eukaryotic gene segments are translated in any of the systems. It remains unknown, although it looks like there are some suggestions of positive data. And I wonder if, on the basis of this, it might not be feasible to consider issuing guidelines in pieces, that is, as information is available, and is met with some confidence by the entire community that you issue that, and that you hold off, which essentially is now, with the requirement for EK2. You would hold off the eukaryote experiments until some of the risk has been ascertained.

Now, I realize that if you don't have the guidelines, who is going to run the experiments to determine the risk? That, of course, comes right back to NIH. And I think this will be a very difficult decision to make--that is, should you allow NIH to run the time requirement, et cetera, without guidelines, any internal guidelines, and then be able to write better prescriptions for these areas, unknown areas? Or, do you write a set of guidelines now in these unknown areas so that you can let the information out on contract and get more participation of the expertise?

I think that is a very difficult decision, but if anything, at this point, I think to let the NIH do it with the advice and expertise of all the people in the area to design the critical experiments.

Again, I mean, I am not a nucleic acid chemist, and I don't feel that my advice on whether it is EK2 or EK3 or something is really worth concern.

I do think that despite all the rules you make, the image is important, and even to scientists the image is important. I would hope that out of this can come an image of responsibility--I am sure it will--but also an image of fair play, and that means to me, anyway, not just fair play on who gets the grant money and this sort of thing, but also a realization that every time you put in rules and regulations it costs money, and this means that already you start an elitist system, because there are institutions who have more money, and there are scientists who get more money, and there are groups that can set up facilities, and there may be a single investigator at the University of Kansas who has no one to do this and no one to support a biohazard thing.

I would hope that somehow in arranging these facilities and the rules you might take a leaf from a physicist who did it in the atomic energy installations after World War II, and make some shared facilities, for

example, where young people could apply and the committee could decide which would have the best priority and should be done, and that these would be available to scientists who don't have as prestigious support of a major institution with funds that can be put into building these. Because in the long run, the compliance in this country with the rules and with the guidelines is going to depend on the image, I think, much more than the actual guidelines per se.

DR. FREDRICKSON: Dr. Zaitlin?

DR. ZAITLIN: Well, as with others before me, I have been scratching out things that I was going to say, but I feel as the only plant scientist on the committee, I can address a few remarks to that. Now, plants are certainly to be concerned with, and there are some rules in the guidelines concerning plants, but I would submit that you probably had a minimal input with respect to plant work. Now, NIH normally has not been a heavy supporter of plant work, but other agencies which will follow these guidelines if they are adopted certainly will.

I am not talking about situations where plant DNA is incorporated into bacteria. These situations are already covered quite well by the guidelines, but I am talking about those situations where foreign DNA of various origins ultimately will get incorporated into the genome of higher plants.

Now, the guidelines specify conditions for the containment of cultures, of cells, organelles, of plants parts during their treatment, and they also state that cloned DNA used in these experiments should be tested to determine their effects on these plants or plant parts.

Now, containment here I think is an easy situation. We are talking about organisms or organelles which, with much lower replication rates, they don't form aerosols, and the cultures are very fastidious in their nutrient requirements.

My concern, rather, relates to what controls are necessary once we have obtained plants with the modified genome containing the desired characteristics. I should add parenthetically that we are certainly a long way from that goal. We need procedures, an outline of procedures to determine if in addition to the introduction of the desirable characteristics, we inadvertently have created an organism with undesirable characteristics.

I feel, therefore, that we need to include in the guidelines restrictions on the release of these plants from controlled greenhouse or controlled growth conditions until they can be adequately tested through several generations. Two specific properties come to mind.

The first is, Do these plants also contain substances toxic to animals that might be likely to eat the plant or its fruit or its seeds?

Secondly, Does the plant have a competitive advantage over other plants and become a pest in itself? Taking as an obtuse example, suppose we were able to create the nitrogen-fixing grass plant. Well, will it become another crab grass, or Johnson grass? I think we need to test these organisms in a controlled manner under conditions where they can still be contained before we release them to the biosphere, where once they are out it may well be too late.

DR. FREDRICKSON: Dr. Callahan?

DR. CALLAHAN: For me this problem comes down to a rather classic case of how does one establish proper policy bias? By bias I mean an inclination to go one way or the other. It seems to me here is the perfect example, at least as a non-expert listening to arguments. I feel my problem is not to somehow stand back and be objective, because I don't know where the base for objectivity lies. In particular cases, yes, but the base for objectivity concerning the possible benefits and possible harms it is very hard. One has to be imaginative there, and hence the question is, What ought to be the policy bias?

Now, I have heard nothing in two days to suggest that there is any moral obligation to do this research at all, that somehow we ought to do it, that somehow we would be remiss to society for the future not to do it. I have heard lots of arguments that it would be morally desirable to do it, morally permissible to do it, morally commendable to do it, but not morally obligatory to do it.

I think there can be no moral obligation to do this research. It seems to me that there is, however, a moral obligation to do no harm. That is a much easier case to establish, that one ought not to hurt other people, or run the kinds of risks which put people in danger.

Now, I think, given the uncertainty in this case, given the fact that one ought to do no harm, but that one may try to do some good, one probably ought to incline in the direction of caution. Namely, the benefit of doubt should go to the worriers, and not to those who are optimistic that problems will be solved. If it seems to be a toss-up of technical opinion, if it seems to be an unresolvable argument whether the worriers or the non-worriers are right, I would say give the bias to the worriers.

It seems to me as one goes thorough the particular elements of the guidelines that might be a workable principle to maintain.

Now, I might, finally, having said all that, say I think that the guidelines are going in the right direction. I would not want to push the

point of give the worriers the benefit of the doubt too far. It seems to me that it is possible to be an excessive obsessive worrier. We all know anxious people, people that are too anxious. In this case, I think the guidelines are suitably worried, if you will, suitably give benefit to the possible harms, and in that sense I am rather happy with them.

DR. FREDRICKSON: When I think of your remarks and Dr. Koshland's I am reminded of a statement of Alfred North Whitehead that style is the ultimate morality. You have referred to image, and perhaps in this case that is somewhat applicable, but I think we will probably not depend upon that alone as far as making a basis for morality.

Dr. Kelly had to go, and he will send us his comments in writing. So that brings me to Dr. Melnick.

DR. MELNICK: I would like to use the microphone, if I may.

I have been concerned with procedural questions. I think we have heard the pros and cons of the science the last 2 days, and I think it is going to be up to the technical committee to develop this further.

Because of the important but uncharted areas opened up by the recent developments in recombinant DNA research, the following procedures are recommended to be administered by the office of the Director of NIH.

The Director shall appoint a technical committee on recombinant DNA to be made up of 15 experts in the field. At the beginning, five will be appointed for 1 year, five for 2 years, and five for 3 years. At the end of each year, five new members will be appointed for a 3-year term. The committee will meet every 4 months. Its functions will be as follows: one, to review the current regulations euphemistically heretofore called guidelines, and to suggest modifications as new information becomes available; two to plan model experiments to be carried out in the appropriate NIH containment facilities. These experiments will be planned to yield data to determine the next steps that can safely be taken to advance our knowledge of recombinant DNA. The data and their analysis shall be published as each forward step is taken. Three, to review recombinant DNA research grant and contract applications submitted to NIH and to evaluate them for scientific merit and for adherence to safety standards.

The technical committee will report to a recombinant DNA advisory council, which serves--which will serve as a policy-making body. It receives reports and, after deliberation, recommends actions to the Director of NIH. The Director appoints the members of the council to be made up of eight scientists, not working directly on DNA recombinants, plus four representatives of the public at large. The eight scientists will be drawn from leaders in related fields of biomedical research, such as genetics, bacteriology, virology, botany, infectious diseases, public health, et cetera.

The four non-scientists from leaders of areas of public concern such as bioethics, law, sociology, medical history, et cetera.

Each institution receiving NIH funds will be required to appoint a local committee on biohazards, which will be expected to become familiar with the recombinant DNA safety regulations promulgated by the NIH technical committee and approved by the advisory council. No research grant or contract will be considered by NIH in the area of recombinant DNA unless the local committee certifies to the satisfaction of the technical committee or its designee that the principal investigator and the local institution meet the safety regulations. If the grant is awarded, the local committee will periodically inspect the laboratory of the principal investigator and submit an annual report of these inspections of NIH to assure that the regulations are being carried out.

Except when the technical committee is considering applications for research grants, its meetings shall be open to the public. Time will be provided to hear the opinions of scientists and non-scientists who feel that the regulations should be modified. These discussions will be summarized and evaluated by the technical committee for consideration by the policy-making advisory council.

DR. FREDRICKSON: Thank you, Dr. Melnick. You are going to send the details of your suggestions to me in writing, aren't you?

Dr. Walters?

DR. WALTERS: Up to this point the field of ethics has dealt primarily with ethical problems arising from clinical medicine and in research involving human subjects. So it is quite a new area for the field of ethics to be looking at problems arising in basic research.

One way of looking at the current proposed guidelines is to compare the guidelines with the letter of the committee on recombinant DNA molecules published in Science in 1974. There, two kinds of experiments were to be deferred--type one, having to do with antibiotic resistance or toxin formation, and type two, the use of foreign DNA from oncogenic or other animal viruses.

It seems that what has happened at the Asilomar meeting and now in the various versions of the guidelines is that there has been a sifting process, and that these two types of experiments have been graded into levels of risk. Still deferred are experiments that would form toxins. Secondly, the use of foreign DNA from oncogenic or other animal viruses is permitted, but under very stringent safeguards. And thirdly, antibiotic resistance: experiments involving antibiotic resistance are allowed to go forward under stringent, but somewhat less stringent safeguards.

In addition, the committee in working through the various drafts of the guidelines has added other types of research which are to be deferred until the risks have been identified. As a lay person reading through the guidelines, it seems to me that the position of the committee is least clear on the question of antibiotic resistance of all these types of experimentation. I would point in particular to pages 11 and 28 of the current version of the guidelines.

On the procedural question, it seems to me that there are a number of resources available to the committee. The rich experience of NIH with institutional review boards, the work of the National Commission for the Protection of Human Subjects, and the work of the Secretary's Task Force on the Compensation of Research-Related Injuries. I would have three comments in the procedural area.

First of all, the institutional biohazard committee: I would echo what Dr. Shaw has said about the composition and function of such committees. I think it should also be made more clear in the guidelines how this committee will relate to the institutional review boards.

Then there is the ticklish question of the monitoring of research in progress. Will such a local committee have any functions analogous to the function of a radiation safety office?

Secondly, it is stressed in the guidelines and also in the English Ashby Report that there should be epidemiological monitoring of all laboratory workers.

Thirdly, in the area of dealing with problems which may arise in the laboratory, I think that perhaps the committee should look into the question of what health insurance coverage would already be available for persons working in the lab who might be injured, and also the question of the compensation of persons injured through their participation in the lab.

Finally, it seems to me that in addition to the kind of safety concern which we have been focusing on here during these 2 days, there may be the need for some kind of ongoing forum which would take up a different type of discussion, perhaps at annual intervals, looking at the progress in the field and also trying to look forward to the potential applications of this research and their possible impact on society.

DR. FREDRICKSON: Dr. Petersdorf's chair is empty. He has promised to send us his comments, which brings us to Mr. Hutt:

MR. HUTT: I would like to premise my specific thoughts with three general comments.

The first is that I am not as persuaded as Dr. Callahan that there is no moral obligation to go forward with this research. In the law certainly there are concepts of obligations to act in order to prevent harm as well as to refrain from action in order to prevent harm. I think this may be an illustration where inaction could be of greater detriment to the public than action. I am not sure that all of us have enough information to really make that judgment at this time, but certainly I am persuaded that it would be wrong to refrain from going ahead at this time as long as reasonable controls do exist. I view that, I must confess, as the principal issue.

Second, the scientific community deserves enormous praise. I am speaking as a member of the public, and I feel very strongly about this, for bringing this issue to the fore. People have said it is unprecedented, and I certainly agree with that. If Dr. Berg and his colleagues do not receive a Nobel Prize for Medicine, then they should receive it of Peace.

(Laughter.)

Not to say there has not been controversy, but the process that he and his colleagues set in motion, which has resulted in our being here, deserves all kinds of public recognition which it may never get, at least not adequately.

My conclusion is that having taken the initiative, the scientific community should be allowed to keep that initiative in attempting to control the proper use of these research techniques. My fear is that having taken the initiative you may not take enough steps to keep that initiative. That is the basic thing I want to discuss for a few moments.

I would like to touch briefly on issues of substance, but primarily, Don, I will send these to you in a letter.

I think there is a question of the physical containment, and that much greater attention must be paid in the guidelines to the limitations on physical containment. I consider the use of the SV40 as just extraordinarily unpalatable to the public, unless it can be explained in much, much, much greater detail, and all of the statements I have heard yesterday and today about this being relatively innocuous are documented with reference to the scientific literature in a terribly unconvincing fashion.

To use any virus that produces cancer in animals, in test animals in a way that is not subject to the most severe limitations, this raises all kinds of questions in my mind, and I am certain in the minds of the public.

I would like to see serious consideration given, both to NIH emphasis on development of the EK2 and EK3, and indeed, even approaching the concept

of the Boston Group of a 2-year limitation in order to force that type of consideration, because if there is no forcing mechanism it may simply occur 5 years later than it otherwise could.

Now, there are conflicting views on the E. coli. I am not capable of even addressing those. I think it important, as Judge Bazelon said, that when you make your decision on whether EK1 can be used for a short time at least, whether there should be a forcing mechanism to go to EK2, whether E. coli should be used in the future, that all those reasons be laid out in enormous detail so that the people on both sides of the issue see your full explication of the basis and the rationale for your decision.

I do feel also that the guidelines need much greater public participation in the decision-making at two different levels. One is at the level that Dr. Melnick discussed, his proposed council. I am not sure that a second advisory committee at NIH on this issue is necessary. I think it could be melded with the basic technical advisory committee.

The second is at the local level: I feel that the limitations on the local biohazard committee, which I believe is on page 44 of the guidelines, are inexplicable. We unfortunately did not have time to discuss that in any detail, and perhaps we could do that at a later time, but certainly I did not see the rationale for them.

DR. FREDRICKSON: Excuse me, here. You mean you refer to the limitation of their decision-making to safeguards as opposed to scientific--

MR. HUTT: Absolutely, and also, as opposed to the broader issue of public perception of safety and including a layman on the committee as is done, for example, with the informed-consent peer review mechanism who can also make a contribution albeit a nontechnical one.

Going to some of the more legalistic or procedural aspects, certainly it is a legal fiction and perhaps also science fiction to refer to these as guidelines. Any document which is going to be used to determine whether a scientist can get Federal funding for a specific research project is not a guideline. It is a rule; it is a regulation; it might as well be a statute.

If it is psychologically useful for the scientific world to believe these are guidelines, why, I have no objection to calling them that as long as no one takes it very seriously.

Now, I personally would go along very, very strongly with what Judge Bazelon said about the need for public procedures to the extent of putting these in the Federal Register. I do not believe that the public rights--whether they are the rights of scientists or the rights of citizens or whoever, and I don't make a distinction between scientists and citizens, but members of the public generally--should be affected by guidelines drawn

up by any particular group that have not undergone the public procedures in the Administrative Procedures Act which affect all of our rights. I therefore believe that while these should go into effect on an interim basis before a Federal Register publication, that they should definitely receive the scrutiny and the protection that goes along with Federal Register publication.

Now, this leads then to the next question, because many scientists and members of the public erroneously believe that if you put anything in the Federal Register it is cast in iron for all time, it is impossible to change or at least very difficult, it will slow down scientific progress, et cetera. I have heard those arguments all the time I was in the Food and Drug Administration.

First of all, and I believe my comments to Dr. Brown of yesterday dwelled on this, albeit only briefly, there are two mechanisms for change. One is, of course, the amendment of the regulations themselves. All of you will say that takes time, and I agree. Therefore, that in itself is not sufficient to provide flexibility. But it is also possible to use what I referred to yesterday as the variance mechanism.

A procedure can be built into regulations, assuming that these guidelines were to become regulations, which would provide that anyone wishing to have Federal funds could request a grant, and in that request for the grant could also request a variance for good and sufficient reason that would have to be spelled out in the request, and that the advisory committee, the technical advisory committee would then advise the Director of NIH whether there are sufficient grounds to assure the public safety and to grant the variance at the same time in order to assure a public record. This is a procedure, I might add, which has been used in regulations before, and I could provide models for this.

In order to assure public awareness of this, the request for the variance and the written grant or denial, which can be relatively brief, would be then placed on the public record so that anyone who wished to be aware of it could see that decision.

Now, a variance can be granted on an ad hoc basis; it could also be granted on a broader basis while the regulations themselves are being revised. I urge all of you not to accept the stereotype that regulations are impossible to deal with and rigid. They can be as flexible as the limits of the English language. For that reason I do believe that they should go in the Federal Register, and that a system of flexibility be worked out.

Now, Don, I have a number of specific problems with the guidelines as written. I will simply sort of list them, and then give them to you in greater depth at a later time.

There is great need to clarify the difference between "shall" and "should" in these guidelines, and I won't dwell on that, because I perceived it as different from the drafting committee, and I think any lawyer would.

There is a need to include things from the minutes that were not included, such as what happens in the procedural mechanism for determining what happens to the existing material that could no longer be used.

There must be clarification of how EK2 gets approved. We went into that yesterday.

Frankly, I think I will simply omit the rest on this list in view of the limits of time, but there is some drafting work that seems advisable.

Let me talk now about probably the issue that more people have raised and expressed concern about than any other, at least those of us from the public sector, or the private sector, member of the public.

(Laughter.)

We are all worried about proliferation of laboratories and experiments and loss of control over this entire process. No one is concerned, I think, about the laboratories that are represented in this room from the leading institutions and medical schools, and so on. We are concerned--and it was crystallized in the form of what will happen in the local high school laboratory. Perhaps it was unfortunate that we used that example, but that was intended simply to illustrate a concern about the problem.

These guides would only be enforceable, whether they are regulations or guidelines or laws, they would only be enforceable by denial of NIH funds. They would not apply to industry, to foundations, to high schools, to many, many colleges and other institutions. I think it highly unlikely that the American public would allow that situation to exist in light of these concerns.

If these go no further than that, and if the work of all that has gone forward to date rests at that point, I think it highly likely that Congress would be forced to deal with this issue. The question then is, is there something further that could be done to broaden the scope of the regulation without opening up Pandora's Box of a rigid governmental control at all levels of development in this area.

Don, I am sorry, I am going to go on a few more minutes. I realize I am taking more than my allotted 5 minutes.

Now, there are various regulatory models in my judgment that could be considered. At one extreme, and I will say I personally do not agree with it before I describe it, would be the type of control exercised by the Food

and Drug Administration over investigational new drugs in humans, where there is a very elaborate procedure which requires submission of a plan every time any experiment is carried out, and governmental approval of that plan beforehand. I am not persuaded that that is necessary in order to exert adequate control in this field.

But what would be a possibility? One would be to try to get the Federal guidelines imposed in the same way that the Equal Employment Opportunity requirements have been imposed throughout the private sector, namely by saying to anyone who has any contract with the Federal Government that they must follow these requirements. That has been fairly successful. I must confess I am not sufficiently familiar with the legal basis for that, that I could give you much comment on that, but I certainly think that is one possibility.

A second would be to set up a central agency in NIH or the National Academy or some other group to monitor on a voluntary basis all research. I find that a little more difficult. I think it would be somewhat clumsy and subject to the same difficulties as the guidelines themselves. It would be a bit too voluntary.

One other possibility which I think does have some promise would be to use a somewhat obscure provision of the existing law, which I am sure most of you are not familiar with, and I brought along a copy to illustrate how it could be used, is Section 361 of the Public Health Service Act, which was enacted many, many years ago to control communicable diseases. After all, what we are concerned about here is the possibility of unleashing something which would be contagious.

Now, I will just read one sentence from this. "The Surgeon General with the approval of the Secretary is authorized to make and enforce such regulations as in his judgment are necessary to prevent the introduction, transmission or spread of communicable diseases from foreign countries into the states or possessions, or, from one State or possession into any other State or possession." And for this purpose he can promulgate whatever regulations are necessary.

It is a very, very broad, ill-defined provision. It has been used in the past in a variety of situations, all the way down to and including the Food and Drug Administration's regulation of pet turtles in order to prevent Salmonella. But it has never been interpreted narrowly, and in my judgment would allow one of two possible means of control if HEW wished to use it.

One would be to license, require licensing of all laboratories engaging in this type of research, and that licensing would be conditioned upon following what we now refer to as the guidelines, which obviously would have to become regulations.

The second would be to license the--and I will probably not use the correct technical terminology--but the cutting enzyme, whatever that scientific word for it was, and the source of the foreign DNA--I hope I have got that one right--that is of concern to scientists. The agents that were described, for example, as CDC classes 3, 4, and 5, and make sure that anyone who was to purchase those for use in any kind of experimentation of this type would again be required to follow the NIH guidelines.

Now, these are two possibilities or three possibilities I have thrown out. I don't see how any of us could possibly do more than begin a consideration of those today, and perhaps even that is not possible.

What I am suggesting is that to keep the initiative in the scientific world rather than to allow it to escape, and to become overrun with regulation, that this issue must be addressed very realistically and very immediately. And I would simply suggest, Don, that the best process should be put in motion as quickly as possible, so that the concerns of those of us who represent the citizenry at large can be reduced to the minimum level.

Thank you.

DR. FREDRICKSON: We will pause, Ms. Haygood, before we come to you because the mail has to be delivered. This is the only place in town where you can count on the mail.

(Laughter.)

I would also comment in this hiatus, for the record, that the empty chair next to Mr. Hutt is that which was allotted to Mr. William Smith, an attorney for the Children's Defense Fund, and Mr. Smith was unable to be with us during this day-and-a-half meeting.

I don't think that you are as interruptive as you think, and I believe we can go ahead and ask Ms. Haygood to comment, if she would not mind.

MS. HAYGOOD: I have quite a number of specific comments arising out of this day-and-a-half meeting, so I--

DR. FREDRICKSON: Ms. Haygood, would you like to use the podium?

MS. HAYGOOD: I have a number of specific comments that arise out of this day-and-a-half meeting, but given that our time is running short, I think I will just hit a few highlights and submit the rest later.

In the area of physical containment, my own personal experience as an itinerant student wandering from lab to lab leads me to conclude that P1 and P2 cannot really be depended on for physical containment as it is generally practiced.

Now, I think that some suggestions that have been made in the direction of imitating consent procedures possibly provide some greater assurance of adequate training, some sort of certification, because I think that we must be assured that everyone who is dealing with these kinds of organisms is aware of the danger, and also has been really adequately trained in how to deal with it. I have seen too many examples of laboratories where there is a general knowledge of proper handling of dangerous materials, but where there was too much looseness and too much flow of people for them to be really adequately enforced.

In the area of biological containment I think I have already commented on my uneasiness about E. coli, and I am even more uneasy about how things are going to be--how switchover to another organism can be implemented.

I think that along the lines of what Dr. Brown was saying in terms of he wanted to see sort of individual consideration of research projects, I would like to see, as soon as EK2 systems are available, that they become the standard laboratory organism, and any experiments that cannot be done with EK2 that need an organism that is of a different genetic composition, special petition or consideration of that research should be given, and as soon as EK3 organisms are available, essentially that means that an EK2 organism becomes an EK3 organism, so some of the distinctions in the guidelines about reducing downshifting from EK3 to EK2 are meaningless in ongoing research.

The distinction about translation versus non-translation of genes inserted into new organisms, I think, can form the basis for further categorization of experiments on the basis of danger, because unexpressed genes are obviously not going to be anywhere near as dangerous as expressed ones. However, I think that this kind of categorization can't take place until after the reasons for nonexpression are better understood, in other words, if circumstances arise, either genetic or environmental, that could cause expression of genes after they escape, I think that has to be taken into consideration.

I think my main concern is the question of flexibility, because I think that ideally if we could be assured that we could recommend the most stringent guidelines that we feel comfortable with, and that they could be recommended as actual estimations of hazard are arrived at, I think that we would all be a lot more comfortable with the situation. It is the idea that these guidelines could become rigid and inflexible. It makes people nervous about coming down too hard. That is why I think that probably the most important task that you have ahead of you is devising methods by which these guidelines can be constantly revised, as information comes in.

Furthermore, I think that they should not be solidified, or ossified, until after experiments, perhaps done by the NIH, give some real estimation of hazard.

I think that the composition of the committee concerned with this ongoing review, which it seems is assumed to be the Program Advisory Committee, should be reconsidered, anyway. I think that most of the comments that I have heard in criticism of the composition of the committee have been in the direction that people seem to think that there are too many scientists actively involved and concerned in the research. I think that situation should be remedied, not necessarily because the work of the committee has been inadequate as a result of that, but simply because this question is going to be raised again and again and again, and I think that for the sake of everyone's conscience and comfort, this question should be dealt with as soon as possible.

I think that Dr. Brown's suggestion that expert testimony be used in decisions is a good one in that it allows for a possible separation of expertise from the body of the committee, and allows that the expertise that is currently represented on the committee can still be available for its use.

My main concern is not so much the details of the guidelines, which I assume are going to change over the next few years, although these are the true substance of what we are discussing here, but the process of their ongoing evolution, and I think that the mechanism by which this evolution will occur is probably the most important--devising these mechanisms is the most important task ahead of us at this point.

DR. FREDRICKSON: Thank you.

Dr. Dodds?

DR. DODDS: I believe that we are fast approaching the expiration of viable comment concerning the pitfalls of the committee such as this, and many good things have been said. I agree with Dr. Melnick in his council. I agree with Ms. Haygood on her requirement for appellate mechanisms. I agree with Mr. Hutt on containment.

I would only caution the absolute necessity for the maintenance of the scientific community, to keep the responsibility for moving forward. In my own profession I have seen what mandated rules can do with respect to quality assessment, which is a biohazard, if you really want to bring it down to the bottom line. It relates to people, and in the PSRO area we have seen an absolute lack of responsibility--well, I shouldn't say absolute lack, but there has been close to an absolute lack of responsibility in this area with local committees that function autogenously and without any real rule from those who know what can be expected or what is to be expected. Believe me, the importance of the scientific committee being responsible can't be overstated.

DR. FREDRICKSON: Mr. Ladwig?

MR. LADWIG: I don't know how many people have seen the movie The Thing. It was a science fiction movie that James Arness rose to stardom in, and in it there was a mad scientist who wanted to go ahead with this experiment that he had cooked up in the lab in growing these organisms, despite the fact that his organisms had escaped and were killing people and this type of thing. He was doing this, he said, in the name of science, and in the name of truth.

I joined the rest of the audience in applauding when he got his just due and was zapped by The Thing, and with that in mind I was very glad to participate in this meeting with scientists who are not mad, who recognize their responsibilities and have taken this first step in managing these experiments, proposing these guidelines, and giving us something to build on.

I believe too many Americans still have a bad image of science. You have been thrown in the same bag in a loss of faith with the politicians, government agencies, the military, and this kind of faith has got to be restored. You are thrown in with a track record of controversial things like the SST, nuclear power, ozone layer, and I am not making any value commitments on these things. I merely am saying that this is the track record you are identified with, and this has caused a lot of alarm.

So my specific recommendations on the guidelines are going to have to come later. My own membership, which I kind of feel I am representing here has been sending me their comments based on a letter I sent them, and it is split right down the middle at this point, so if I am allowed to feel like a mini-you for a while, I have got this slush coming from both sides. I may just give it all to you like that and not make any judgment at all.

(Laughter.)

So I just want to make a couple of quick comments and concerns at this time. I agreed with Dr. Rosenblith, yesterday, in his assessment that we are not dealing here with an either/or situation. This isn't an adversary situation. We should certainly be seeing this from many aspects, from the legal implications, to economic implications, government, theology, politics, and I don't think enough people in this room have been talking and realize that point, that as we are moving into what has been called participatory democracy, and it is coming, and it is going to come stronger in the next few years. If the scientists feel that the public is meddling in this type of thing, just wait, because it is going to get worse until we find a system to manage participatory democracy.

Scientists are involved in other people's bailiwicks, and this type of thing, so we are all getting our fingers in each other's pie, and until we can find a way to manage that system, we damn well better learn how to go on with this type of forum.

I think Dr. Fredrickson should be commended as being a person in what our members refer to as being a bureaucrat, as taking that step to include a broad public participation. So that is the first point, realize that participatory democracy is coming, and you can pat yourself on the back for this first step you made, but keep looking over your shoulder.

Okay, it has also been brought up who should make this decision. Should Dr. Fredrickson be the one making this decision? Is that too much responsibility to give one person? Congress and the President are fighting about this thing right now. Congress wants just a little more to say about policy than the President is letting them do. I think this is an irrelevant question at this point, because this is the system we have right now to work with, and I think we need to support Dr. Fredrickson with as much information and opinion as we can give him, and for the time being we have got to live with this system.

The third concern is the military applications of this thing. Our group also works on aerospace activities, and we have been trying to find out for three years what the military is up to in outer space. We can't get any answers there.

I don't want to have that same thing start happening in this area, and I think the scientists and--I don't think we can do it with these guidelines, again, because NIH is just part of a huge picture, but I think we really need to stress that the military also adhere to these guidelines.

Okay, another point is what kind of budget are we really talking about here? For fiscal year 1977, research and development got increased substantially, but do we know that that is going to happen the following year? There are only eight science-oriented Congressmen in the House and Senate, and that is not very much in the favor of scientists to express your point of view. For all we know, in the next couple of years down the line, if we don't get this type of issue discussed in the public, you won't get any budget money for this type of thing, and you can use your pipettes to pick you teeth, because that type of thing isn't going to be there.

So I want to keep stressing, let us continue this public input, get this discussion going, so we don't have to get down to the emotionalism and fights later on down the line.

Okay, another point is the physical containment. I feel that we have been given a bit of a snow job on this, and on paper that looked real nice, all those different facilities. But the human factor is involved here, and you can't tell me just because at P3 a facility is going to--has got all these safeguards. There are still humans involved in that. I have some contacts at the lunar containment facility who spent many times more developing that than any of these facilities we have been discussing, and they told me that they had stories that would knock my eyes out. Well, I don't

want my eyes to be knocked out on this, and I think we need to come forward with any of these stories ahead of time, and realize that we can talk about physical containment, but some scientists do get a little sloppy sometimes, no offense. But we need to do something in these guidelines to make sure that our actual laboratory conditions and things are being checked up on and are not being sloppy.

There has been a lot of talk here about we don't want any risk involved in these things, and I can't buy that 100 percent, because this is not a no-risk society. There are risks in anything you do. The coal miner is risking his neck, the guy building a tunnel somewhere is risking his neck.

So I think that point is being over-emphasized. I think we need to look here at a minimal risk, and I agree with what everybody has said already, let us clarify what those risks are.

My question on what is the punishment for not following these guidelines, which Dr. Dodds also brought up still hasn't really been answered. I don't know, are we going to get into a new era here where we are talking about white coat crimes along with white collar crimes? I don't know what the answers to these are, that is why I am asking. I think we need to get these things squared away. Is somebody going to hold back on some research that he did that is beyond the guidelines and wait until those guidelines are modified and then jump out of his lab and say hey, look, and get his Nobel Prize or whatever.

So I would like to see that punishment thing looked at a little bit more.

I very strongly do recommend this review clause. I think that that is a great thing built in. As part of that review clause I think every group that is represented here today, and I noticed a big effort was made to include public interest groups, I think now is the time for all of us to start working on the programs to discuss this type of issue in the public sector, find out what the concerns are, don't present it from a one-sided point of view, but hold forums to discuss this thing, and show all the different points of view so that when the next review process comes up you will have even more public input, and even more of what I would say a glory for the initial thing you started here.

My final point, then, is that I think we should all be working here to establish bioethics as perhaps a new standard much like the space program became for coffee. We want to look at bioethics as being a standard to look up to, and that can be applied to other things.

We comment on the space program, and everybody says well, if we can go to the moon why can't we get a good cup of coffee. Well, that is because the space program has become a standard of excellence, something to look up

to. I think that this area we are talking about here, with the effort that has already been started by the people three years ago or however long it has been, should become a new standard of ethics for not only things in the scientific community, but in other aspects as well.

DR. FREDRICKSON: Mrs. Peterson?

MRS. PETERSON: I think what I can say after his and after Peter Hutt's, is Amen, because they have touched, certainly, as around the room, on so many of the things that I feel very strongly about. I would like to just underline a couple of those points.

I think maybe I would like to start off with maybe an item that should cheer us, and that is that maybe the young, as you said, the nay-sayers, and I can speak of this because I belong to the gray-haired generation, are the ones that are giving us a new social and moral commitment to these things, and I think that should cheer us. I for one welcome it, and welcome that type of participation.

In the whole question that Mr. Ladwig raised about participatory democracy, as we go around and feel the mood of the country, people want to know what the trade-offs are. They want to know what our priorities are, and many of them are asking these questions: Well, how much is this going to cost? And is that from the public point of view a legitimate expenditure in relation to other kinds of priorities that we may have?

I think along with the many good questions that have been raised, I would just like to touch on a couple of them, because I have checked most of them off.

But I want to be absolutely sure that the guidelines and whatever develops, which I know will be well in balance, do cover as wide a range as we can. I have had some experience with the granting of Federal contracts, and I know grants, you have a rather tight control, and I want to be sure, Peter, that in the guidelines that you are working on that this goes as broadly as it can. I know many areas where we have let them out, and we don't have the controls on that.

I would like to be sure that the authority that has developed somewhat along the lines that you describe is extended. You talk about the Defense Department. Well, what about the Department of Agriculture? What about all the others that are there?

The part that I am really concerned about is getting into the private sector. What kinds of guidelines and controls can we get there, as well as Defense, industry, and these areas?

I am concerned in those areas about what we do about quality control along the line. Who makes the decisions for quality control? Who says at what point is this hazardous or not hazardous? I think there are the things that we are very, very concerned about.

Another is how do we recognize and how do we control the purchase of the substances that go into the experimentation, the enzymes? I am so aware of the mood of the country relative to the questions in the radioactive field. What do we do about registering? How do we know who is buying these things? How do we know where that is going? I would just like to see that we--I would feel more comfortable if we could feel that we are factoring those questions into all of it as much as possible.

Again, a little knowledge is a dangerous thing, but you talked more about the antibiotics and these things, and maybe we have enough. I wonder if we don't need to look at, again, where the priorities are in what some of this will come.

But finally, I guess, the scientists in this room are thoughtful people. They are responsible people. All who have participated are, and I am positive that the judgment that comes to this will be good. But it doesn't mean that I don't have fears, many of which have been expressed here, about the proliferation. That is somewhat frightening.

It frightens me a little, too, in relation to what I would like to call the mood of some sections of our country. I think the things that you said about beginning to interpret it, to develop a climate of understanding, to make possible the kinds of things that you are working on here to become accepted, we know, those who work with Congress and work with the legislative know that it is terribly easy to get a pied piper issue, I would call it, to get hysteria, to get people moving on it. And you, who have been out at meetings as I have, where you sensed the mood of the people on--let me just take the abortion issue, for example, touching with God. I can just see certain groups get hold of this, and again, my plea is that we continue this participatory democracy area, that we continue so that those of us that go out and make speeches can help develop this, can help develop a climate of acceptance.

So we will move ahead, as I know you want to move, and as I know you will move along. The guidelines must have all the expertise possible, but also all the humanity.

DR. FREDRICKSON: Thank you very much.

Professor Rosenblith?

PROFESSOR ROSENBLITH: Well, there is hardly any time nor is there an ecological niche, but it has rarely stopped the Professor.

(Laughter.)

I think the tone of concern, both within the outer and the inner circle here, and to some extent I think the outer circle is really the inner circle, is obviously something that I resonate with. We're dealing with problems in decision-making and uncertainty, where the people who are best qualified have brought us the task of evaluating what is the cost of reducing the uncertainty. In some ways this goes back to perhaps the statement from Ecclesiastes, that he who increases knowledge increases sorrow, and I think as we have lived in the Western World over the past several centuries with the general ideal of progress, we seem to think that the whole history of science, and indeed, of mankind, supports the view that what Ecclesiastes said ain't necessarily so.

Perhaps what we are trying to design here is to be sure that it ain't so.

Now, many will say that this is a good beginning, where do we go from here? They would like the kind of certainty that I think we cannot give. We can only assure a certainty of process, a certainty to which we have to commit ourselves and to which we have to commit a kind of tithe of our time, because that is what we have to contribute to it. I think that some of the colleagues of Paul Berg and Maxine Singer have given more than a tithe of their time.

I think therefore that this is just a beginning. I feel that I regret that we didn't have a chance to talk about the institutional biohazard committee, because we have to create a much broader community with which to operate. Perhaps because I am impressed with the fact what I have seen our activity in human experimentation operate. I have seen when it came to issues of social research having to do with desegregation that they really had no relevant guidelines and had to invent something else.

We have raised a kind of consciousness about the way in which we do research that has paid off in areas that we had originally not encompassed. I think this is what we want to do here. I think we have to understand that many of these committees will start out in a very different way, and yet they will connect to the whole academic community, and I daresay, beyond, because no longer is the functioning of committees of academic institutions immune from public inspection.

So I really think that the problem of building this broader community is absolutely necessary.

Now, to those who say perhaps we are over-instrumenting all of that, I can only say that as basically a physical scientist I am impressed with the fact that the time constant of making enough survival of physically based

technology is so much shorter than the time constant of what we are about here.

It may well be that this is the only technology that will continue, subspecies, and when you are concerned with that I think you are not likely to over-instrument.

DR. FREDRICKSON: Dr. Hudson?

DR. HUDSON: Thank you very much, Mr. Chairman.

I am sure that we are all very familiar with another conference which spent a great deal of time disproportionately with reference to the shape of the table and the positions of persons thereat, and since I didn't have anything to do with the consideration of where I was going to sit at this table, nor in which direction Dr. Fredrickson was going to take in terms of asking persons to talk, I could look at it on the one hand that I have the best of all worlds, and that is I have heard everybody talk, and since I am the last one to speak I could possibly attempt to summarize what has been said. Certainly there is little I could say that has not already been said. On the other hand, I could get rather paranoid about where I am sitting.

(Laughter.)

I shall do neither of these. I would first like to commend the investigators who have brought us to the point of having to deal with these types of questions, even though they are extremely difficult and offer us a great amount of trepidation with our concern. I think it is important that we are here and that we are considering these questions.

I would also like to commend the four various committees that have worked assiduously in trying to design guidelines or rules, but certainly that have been grappling with the very essence and depth of some very weighty questions.

I would like also to commend Dr. Fredrickson for calling this conference together for the consideration so that we might be able to be more knowledgeable about some of the kinds of things that must be considered here.

I don't envy your position with reference to the decision that you must make, or certainly the decision in consultation with others, but I do say to you that I think the posture which you should take should be certainly conducive to the continuation of experimentation in this particular area, without the investigators feeling a sense of intimidation to the extent of "Big Brother is watching."

I do feel, however, that the regulations, which initially should be set very high, should certainly show flexibility with regard to the acquisition of further knowledge.

I feel the risks are certainly real. I don't think there is anything we are dreaming up; certainly, based on the amount of information we have, those risks are real. But as has been said, we certainly are a risk-oriented society, and I think that they are worth taking.

I feel that the personnel aspect of our total approach to this area is one that we need to pay a great deal of attention to, specifically in the area of training. I don't think that we can say too much about the training necessary, and the quality of training necessary. We know in graduate programs that when one comes down to take his language qualification test, there are certain crash courses that can teach you to speak and read German in a week or two weeks. I am being a little bit absurd here, but I don't think we can afford the kind of crash courses for quality training, as laboratories will proliferate, asking some of these same kinds of questions.

I don't think that we can approach this whole area on the business-as-usual type of posture. I think sometimes we get so involved in our own work, and the drive toward what we feel is ultimately possible sometimes overshadows our own judgment.

Certainly I don't want to appear as an alarmist, but I feel that this mode of experimentation is a total new ballgame, and sometimes it is a little bit difficult to change what might be considered mid-stream, with reference to the fact that it is literally a new ballgame. I think training and control procedures cannot be overemphasized, and I mean control procedures that relate to the total scientific community. Not just the university community, but the total scientific community.

As a pharmacologist, I am often reminded of much of the SAR studies, the structure activity studies that we do in our laboratory where on the basis of extremely good information we decide that we are going to design certain kinds of chemical substances that are going to act on certain kinds of specific disease entities without certain types of untoward effects. In many instances we get the compound with all kinds of things that we never even conceived would be possible. They do happen.

So I think, in conclusion, that we are standing very much at a point where Columbus might have stood facing the Flat World Society, and wondering whether he was going to fall off the edge if he continued to insist that the world was round. I think we must take the concept. We must believe that the world is round. We must sail forth. We must not be with inaction, but we must sail forth with precaution.

DR. FREDICKSON: Thank you, Dr. Hudson.

Several others have asked to speak, but I must reserve the chairman's right to attempt to preserve the symmetry of the meeting, and also to see that you get to lunch.

I began with a quotation from Justice Frankfurter, that you recall, to the effect that the history of liberty is the history of the observation of procedural safeguards. I want to thank the committee, and commend it for stepping forward in the direction of that challenge.

I am very grateful to you, indeed, for the time and effort that you have given us. No less so am I to the experts who were on our agenda, and who gave so much assistance to us, and no less so am I grateful to the members of the public who also participated, many of them--all of them, so far as NIH was concerned--at their own expense.

We are really not an inner circle and an outer circle. We are all one in this matter. I extend my gratitude to you for this instructive and important day and a half that we have spent. All of you are very busy, and you observe that I don't have a minute to lose. So this session is now adjourned.

[Thereupon, at 12:30 p.m., February 10, the meeting was adjourned.]

APPENDIX A

DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS
HARVARD MEDICAL SCHOOL
25 SHATTUCK STREET
BOSTON, MASSACHUSETTS 02115

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November 24, 1975

Dewitt Stetten, Jr.
Deputy Director for Science
Office of the Director
National Institutes of Health, PHS
Bethesda, Maryland 20014

Dear Dr. Stetten:

Being 'concerned' with the "Woods Hole Guidelines" for recombinant DNA molecule studies the undersigned scientists met together during the Fall to analyze this document and research the recombinant DNA molecule problem. We ultimately decided to write out some ideas in detail and these are to be found in the enclosed report.

Boston Area Recombinant DNA Group

Margaret Duncan

Margaret Duncan
Department of Microbiology and
Molecular Genetics
Harvard Medical School

Richard Goldstein

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cc: all members of the Recombinant DNA
Molecule Advisory Committee

The central points in our proposal are the following:

1. Since the potential hazardous consequences are extremely serious, and the probabilities of the hazards occurring are non-quantifiable, worthwhile experiments should be conducted as safely as possible. p. 2
2. We feel consensus is possible to defer performing 8 specific kinds of experiments. p. 3.
3. Ecological considerations concerning procaryotic hosts are more important than any possibilities of genetic modification ("disarming" host-vectors), as far as reducing human health hazard. pp. 4-6.
4. We therefore urge the abandonment within 2 years of E. coli and development of safer procaryotic host-vector systems. pp. 6-7.
5. The real levels of physical containment are P3 and P4. P3 needs precise description to be meaningful. pp. 8-10
6. The recombinant DNA committee can at the December meeting specify the "EK2" host strain, the specific, individual "EK2" vectors, and insure their distribution to all investigators. p. 11.
7. Experiments putting certain prokaryotic strain DNA into E. coli are reasonably done with P1-P2 containment. Experiments putting eukaryotic DNA into E. coli are hazardous and suggest EK2, supplemented by P3 or P4. Several of these kinds of experiments are so hazardous as to be deferred. pp. 11-14.
8. Experiments using eukaryotic host-vector systems are described. P3 and P4 containment, and the use only of well characterized cell culture lines, and genetically modified vectors are supported. Many unknowns concerning plant experiments are raised, and we question the view that lower eukaryotic host-vector systems should be regarded in a light similar to prokaryotic systems. pp. 15-16.
9. Implementation of guidelines will face certain obstacles. Valuable procedures would include a public record of biohazard decisions, biohazard impact statements accompanying grant applications, epidemiological surveillance procedures, and local broad-based safety committees. pp. 17-20.

The social benefits which may arise from DNA recombinant work will be of equal value whether they come in 20 versus 25 years, 50 versus 55 years; 100 versus 105 years. They will endure, if valuable, for many centuries thereafter. For 5 or 10 years now, a slow, thoughtful, research-based approach to limiting hazards makes sense.

Many biologists have worked hard and long to draft the Asilomar Statement and Woods Hole Guidelines. We admire this work tremendously and feel it a very positive development when a community of researchers join together planning to eliminate hazards from experimental design. The initial unprecedented response by scientists to a new technology, calling a moratorium and the subsequent Asilomar meeting, saying there are certain things we are wise enough to refrain from, brought public approval on a wide scale.

Now the situation in practice and on paper is in flux. Our own current position is that recombinant DNA experimentation is valuable in selected instances and hazardous in others. The problem facing the guidelines committee is to choose carefully those few experiments which will lead to societal benefit from the collection of innumerable, possible experiments, many of which will be too hazardous to merit being done. In stating that an experiment creating a new organism is too hazardous to be done, it bears clear emphasis that there is no issue of freedom of inquiry. We fully support complete freedom of scientific inquiry. However, there is here an issue of "freedom of manufacture" - the manufacture, in this case, of self-replicating novel organisms, which are potential global health hazards or ecological hazards.

The importance of care in this manufacturing can be seen from many points of view. One prominent to us which has received low attention is that if a mistake occurs, particularly the development of a novel human pathogen, there might well be justified public outrage. This in turn could threaten continued government funding in many areas of basic biological and medical research. This element of self-interest of researchers in all areas should be borne in mind along with the other pressing reasons for insuring that the experimentation is done in a safe way.

The difficulties of estimating by theoretical considerations the potential hazards of DNA recombinant experiments appear insurmountable. A comparison might be made to the dangers involved in using radioisotopes in biological research. With radioisotopes the direct dangers to lab personnel, dangers of disposal into the water supply or into landfill, etc. can be quantified. It is known that exposures to certain levels of radiation is dangerous, whereas exposure to certain lower levels is not. It is thus possible to say that "this level of safety (procedure) is adequate" while "that level of safety is inadequate." The situation is fundamentally different in the case of the recombinant DNA experiments. Here the dangers can not be quantified; they can only be guessed at over an extremely broad range of probabilities (from high to low) for any single, given experiment. It is thus very difficult, indeed somewhat unrealistic, to say "this level of safety (procedure) is adequate" while "this level is inadequate" as the Asilomar and Woods Hole Guidelines have attempted. Rather an inevitable, prudent philosophical approach for this type of hazard seems one which says "we will do those recombinant DNA experiments judged to be worthwhile, in as safe a way as possible." This approach is justified because the potential hazardous consequences of the DNA recombinant work are extremely serious, and because of the all-important distinction between quantifiable (e.g. radioisotopes) and non-quantifiable (e.g. recombinant DNA) hazard probabilities.

The detailed analysis in the preceding paragraph, concluding that worthwhile DNA recombinant experiments should be conducted as safely as possible, leads us to propose a conservative set of guidelines.

A. First of all, in overview of the specific proposals below, we believe consensus is possible in the research community that the following experiments not be done at this time. (Certain detailed reasons and hazards are elaborated in the individual categories listed later).

Experiments not to be performed:

1. Introduction of the DNA from mammals or any warm-blooded animals into E. coli. (this applies to total DNA and "purified" segments, cf. below). The possibilities of introducing tumor virus genomes, enzymes converting bacterial metabolites to small molecule hormones (e.g. adrenaline) and many others, make such experiments extremely hazardous.

2. Introduction of DNA coding for resistance to antibiotics into any bacteria in which this drug resistance does not naturally occur.

3. The use of currently available animal viruses as vectors.

4. The introduction into E. coli of the whole genome or any "purified" segment of a viral DNA which is oncogenic in any species. Also the formation of hybrids of total or partial oncogenic virus DNA molecules with E. coli vectors, whether or not these hybrid molecules are subsequently intentionally cloned in E. coli.

5. Formation of recombinant DNA molecules (and, clearly, subsequent cloning) where any part of the recombinant DNA is derived from highly pathogenic organisms in classes 3, 4 and 5 of "Classification of Etiologic Agents on the Basis of Hazard" (1).

6. Formation of recombinant DNA molecules with DNA derived from organisms containing genes for the synthesis of toxins of very high toxicity (e.g. botulinum, diphtheria toxins).

7. Formation of recombinant DNA molecules where part of the recombinant is from plant pathogens whose virulence or host range might likely be increased.

8. Introduction into E. coli of DNA from higher plants which are known or suspected to make substances with potent physiological effects, e.g. alkaloids, toxins.

9. Any recombinant DNA molecules or strains carrying them mentioned in items 1-8, which have already been made, should no longer be used, but rather be digested, autoclaved, etc..

B. The second item in this overview of our specific proposals involves a discussion of the E. coli K-12 host-vector system and physical containment levels. The suggestion of a "disarmed", low-survival, E. coli host made in the Asilomar report, carrying possible specific mutations enumerated in a footnote of the Woods Hole Guidelines, deserves particularly careful consideration.

This "disarmed" host has not been constructed at this time (11/1/75), at least no paper describing it has been published. DNA from various organisms and viruses is being cloned in E. coli at this time in hosts chosen by the individual investigator. These hosts, insofar as we can find out, do not carry a variety of non-revertible, independently acting mutations which give the host the low-survival, lowered genetic exchange properties of the theoretical "disarmed host." It is essential that the December meeting of the guidelines committee specify one E. coli strain as the "safe" E. coli host, to be used in all experiments, and make it available. This "December '75 disarmed host" will probably be far short of the theoretical ideal "disarmed" E. coli and can and should be replaced with superior strains as they are constructed. It is important that there be at any one time one specific "disarmed" E. coli strain available and used in all cases. Similarly the December meeting can specify the specific "safest" E. coli vectors available and to be used: one mutant lambda vector, one pSC101 vector, etc. This will aid uniformity of precautions on the part of all investigators. However, we do not wish to see continuing for a long time a process of replacing the December '75 "disarmed" E. coli hosts and "disarmed" E. coli vectors with more "disarmed", later model E. coli host-vectors. Rather, we feel that the E. coli system is inherently of such potential hazard that it should be rapidly phased out of use for recombinant DNA experiments.

In as much as E. coli is a normal resident of the human intestine, pharynx, and a human pathogen, its choice as a recipient organism for implanting foreign genes seems reckless. With its universal, intimate relation with humans, E. coli presents a fundamental ecological unsuitability for recombinant DNA experiments. On the other hand, knowledge of E. coli molecular genetics is far more advanced than that of any other organism, offering the theoretical possibility of containment via genetic construction of crippled E. coli hosts and vectors. We

are convinced the ecological considerations concerning procaryotic hosts are more important than any possibilities of genetic modification, as far as reducing human health hazards. Our reasoning follows.

The Woods Hole Guidelines of August spoke of an untested, E_K-2 system where survival of host and vector would be lowered 10^6 compared to wild type E. coli. Suppose, and it is indeed a question, that such "disarmed" hosts and vectors can be constructed, so that survival potential in laboratory media and laboratory rats is lowered 10^6 , or even further. It is erroneous to believe that the nominated "safe" host-vector systems insure "safe" experiments. Over the many years that these experiments will continue, there may very well be genetic exchange from the constructed "safe" host vectors to wild type vectors. This could occur on lab surfaces; it could occur in someone's body following inhalation or ingestion and preceding death of the crippled original host, or in sewage systems if the crippled host survives to be excreted; it could occur in "safe" cultures which are contaminated. Perhaps, above all else, prolonged survival and genetic exchange could occur in special natural environmental conditions sometimes encountered by E. coli, that we in our ignorance of this bacterium's specialized habitats, know nothing about.

We can focus momentarily, for an example, on contamination by both wild type E. coli and wild type vectors of "safe" cultures. Bacterial and phage contaminations of lab cultures are very common occurrences in microbiological labs despite investigators taking extensive precautions to avoid them. Suppose the initial culture of "safe" host is contaminated with wild type host at the time the recombinant DNA molecules are introduced. Then the wild type E. coli may pick up the recombinant DNA and replicate it right from the start. Phage

and bacterial contamination of the "disarmed" host-vector on plates, in liquid culture, during long-term storage and recovery of "disarmed" strains, during shipment from one lab to another, all could result in the transfer of the foreign genes to wild type E. coli. The mechanisms will be contaminant plasmid recombination or mobilization, contaminant phage recombination, and generalized and specialized transduction (possibly by unidentified phages to which the crippled host could not be made resistant, even if it is resistant to some known phages). The converse situation in which the "disarmed" host-vectors become contaminants of wild-type cultures grown by people doing everyday E. coli biology down the hall, offers the same possibilities of "disarmed" survival and genetic exchange, followed by the certainty of entry into the world at large, as such cultures are customarily discarded into open sinks.

Though any of these many possibilities on a given day may be remote, their collective weight over many years and many experiments seems substantial. Any of them will have the same result, that particular foreign genes will end up after awhile in the gene pool of wild-type E. coli, the bacterium indigenous to us.

With all this (and subsequent ideas on physical containment) in mind, we strongly push support for an ecologically appropriate procaryotic host-vector system and the abandonment of E. coli as a host in inter-species recombinant DNA experiments. To insure that this transition be made, the following steps seem

worthwhile:

1. Bolstering immediately, research, meetings, and collaborative efforts of microbial taxonomists, ecologists, and molecular biologists. A wide array of prokaryotic organisms has been searched for restriction enzymes of different specificities to make the experimental technology more versatile, the same can be done to make it more safe. We do not have the background in our group to name an excellent prokaryotic cloning host. B. subtilis does seem superior to E. coli in many respects, although again foreign genes might be transferred from non-spore-forming mutant hosts to wild-type. It also under special circumstances causes human eye infections. An ecologically excellent organism would have a thoroughly specialized ecological niche, geographically distant from laboratories. Thermophiles and halophiles would probably never make foreign proteins, so obligate psychrophiles can be considered on that count. Many other gram-negative and gram-positive species have potential. Research on this matter seems necessary.

2. It should be decided that after two years, that is January, 1978, NIH will no longer offer new grant support of inter-species recombinant DNA studies using E. coli. This would certainly be a stimulus to the search for safer host-vector systems, and place a time limit on the kinds of experiments being done now.

3. It would be naive of us in the molecular biology community to fail to anticipate the immediate use of inter-species prokaryotic recombinants in the pharmaceutical industry. If at this time we further the entrenchment of recombinant technology in E. coli, rather than applying pressure for the development of other systems, industry too will use E. coli. All the problems touched upon above will be much more acute with daily large scale industrial operations; and industry will be interested in extremely hazardous types of experiments, e.g. the manufacture in

prokaryotes of human polypeptide hormones. The development of alternative prokaryote systems is made triply imperative by a peek at the pharmaceutical world.

C. Physical containment levels:

We believe that levels of physical containment are over rated on paper since they are all vulnerable to unavoidable and common dilemmas--human error and carelessness. In the ideal, we would much prefer to rely on a biological system of containment which would render all organisms in question completely impotent and nontransferable outside the milieu of the test tube or incubator. Because of our complete lack of faith in E. coli as the proper system for recombinant studies, we have no other choice considering the biohazards involved, but to rely on strict physical containment measures. We feel that these levels of physical containment should be, as described in the Asilomar report, greatest at the beginning and modified only if and when there is an actual and substantially documented case showing the risks in question to be minimal. Furthermore, we feel it is and should be the responsibility of the researcher who wishes to carry out these studies to provide this substantial documentation for the downgrading of any hazardous or potentially hazardous work. We also feel, that 'potentially hazardous' until proven otherwise should be equated with 'hazardous'.

Four levels of physical containment are presented in the 'Woods Hole Guidelines'. In truth, in what we know to be the everyday reality of laboratory science, it should be obvious that no real difference exists between the first two levels of containment, P1 & P2. The differences between P1 & P2 may be significant on paper, but they will not hold up in actuality for many reasons---apathy, pressure, sloppiness, carelessness--all human attributes. From this we conclude that P1 & P2 should be lumped together as one category, and only accepted

where potential danger does not exist, i.e. only with routine studies with organisms calling for nothing more than basically accepted microbiological practices. Anything of 'potentially' hazardous or hazardous nature should be switched to a minimal level of real physical containment, i.e. where permanent biohazard signs are posted, doors are locked to prevent unauthorized entrance, aerosols are properly dealt with, labs are under negative pressure, etc..etc.. This would be minimal P3 level containment as noted in the 'Woods Hole Guidelines'.

We consider the P3 ('moderate') level of containment to be the first and minimal level of containment for any experiment of 'potentially' hazardous or hazardous nature. Despite its being more stringent than P1 & P2 we still see in it the potential for the human attributes described above to come into play. For this reason we feel its definition as found in the 'Woods Hole Guidelines' makes it quite deceptive and over-rated in terms of actual safety. In addition, the 2nd paragraph in this section of the 'Woods Hole Guidelines' (see page 6, "P3-level research may be conducted in....."), essentially is an option for avoiding the stricter and more reasonable regulations set down in the previous paragraph describing P3 in the Guidelines.

This 'loophole' must be eliminated if P3 containment is to be meaningful. The stature of P3 containment as the most minimal real level of physical containment for any recombinant studies should be understandable in light of our strong opinion as to the inadequacies of the complementing EK biological containment system.

P4-level containment, as defined in the 'Woods Hole Guidelines', should in our opinion provide the security necessary for experiments involving 'highly infectious and or hazardous materials', though as described above we feel that many

such experiments should be all together left undone.

In summary then, we suggest that:

1. P1 & P2 be lumped together as one category ("no risk");
2. P3 be considered to be the most minimal level of physical containment ("minimal risk") for any experiments of "potentially" hazardous or minimally hazardous nature. Since almost all experiments with recombinant DNA molecules can be considered at least potentially hazardous due to the as yet unforeseeable consequences of these studies, almost all such experiments will require at least P3 containment. This would mean that P3 containment would span a much broader range (with a particular broadening to cover experiments previously considered to be placed in the P2 level category in the 'Woods Hole Guidelines'). In addition, we cannot strongly enough emphasize that the 'loophole' presented in the 2nd paragraph governing P3 containment in the guidelines be eliminated.

And,

3. P4 containment be the next and most stringent level of physical containment ("moderate-high risk"). Because of our lack of faith in the 'EK' biological levels of containment we point out here that many experiments previously suggested for P2 or P3 restrictions along with some or another 'EK' restriction would prudently be carried out under P4-level containment. P4 may represent an inconvenience to those wishing to carry out this work. But, safety will have to take priority in the face of unknown risks, potential hazardous consequences of large magnitude, and the current poor choice of the biological system.

PART II. SPECIFIC PROPOSALS

A. Experiments with the E. coli-host vector system

We have above stated two points:

1. The committee can specify one "EK2" strain and one "EK2" vector of each type and set up a lab-office which distributes them to all investigators doing cloning in E. coli.
2. No new NIH grant support for inter-species recombinant DNA studies in E. coli should be administered from January, 1978 on.

Specific proposals for current E. coli host-vector studies:

(a) SHOTGUN EXPERIMENTS (non-purified DNAs):

(i) EUKARYOTIC DNA RECOMBINANTS

It can be taken as a 'given' that recombinant DNA experiments with eukaryotic DNA are the most dangerous and speculative of all recombinant DNA molecule experiments. And, that 'shotgun' experiments, where large numbers of undefined genes are manipulated in a totally 'blind' situation, are potentially the most dangerous of all. Because of this, when they are to be done, such experiments should only be performed under P4 containment.

Mammals - for the following reasons we feel that such experiments should be deferred for an indefinite period:

- 1) the extent of containment possible with any of the suggested host-vector systems remains to be studied and demonstrated;
- 2) A high probability of contamination in these experiments exists with either unknown or undetected 'cryptic genes' or other genes known to exist and code for products responsible for cell transformation.

In addition, there are genes coding for polypeptide hormones and enzymes converting bacterial metabolites into physiologically or pharmacologically active substances, any of which might be produced and leaked out at an uncontrolled rate from the bacterium. The risk involved in such studies is inherently so high that it far outweighs any potential benefit.

Cold-blooded animals and all other eukaryotes

The actual risks here are close to those in higher eukaryotes, though slightly lower perhaps in that C-type virus genomes and polypeptide hormones directly active in humans may not exist. Nonetheless invertebrates such as Anura, the common frog, are afflicted with oncogenic viruses and the potential for conversions of prokaryotic metabolism do exist. Drosophila, for instance, is known to have several pigmentation loci, which involve conversion of amino acids to pigments. First products in such

biosynthetic pathways can be pharmacologically active amino acid derivatives. In the case of the *Drosophila* vermillion locus and tryptophan pyrrolase, the hazard might be small, but in other instances, less is known. For every single possibility like this one can think of, there may be 10 or 100 that one can not come up with, ahead of time. Because of the potential dangers and the leakiness of the biological containment, we feel that such work should be limited to P4 containment.

Higher Plants - the combination of the airborne spread of *E. coli* plus the knowledge that plants produce from bacterial metabolites alkaloids, other toxic compounds, and carry pathogenic agents strongly indicate that all work with 'higher plants' be done under P4 containment.

(ii) PROKARYOTIC DNA RECOMBINANTS

Prokaryotes that naturally exchange genetic information with *E. coli*. P1-P2 type containment seems reasonable if it can be demonstrated in advance that DNA to be 'shotgunned' from such organisms contains no pathogenic properties and that it codes for no known antibiotic resistance markers. Any organism of potentially pathogenic or pathogenic property would call for P3 level containment.

Prokaryotes that do not ordinarily exchange genetic information with *E. coli* - where it can be demonstrated that there is no potential for pathogenic or drug resistance transfer we would still call for P3 level containment due to the unknown hazard built into such experiments. P4 containment should be mandatory for any experiments where the above hazards, though minimal in effect, are known. We strongly suggest deferring all such experiments where strong pathogenicity (e.g. cholera) in the prokaryote has been shown to exist.

(iii) CHARACTERIZED CLONES OF DNA RECOMBINANTS DERIVED FROM SHOTGUN EXPERIMENTS

In the case of cloned DNA which has been purified, shown not to contain harmful genes, and is derived from an organism naturally exchanging DNA with *E. coli*, we feel that P1-P2 type containment is sufficient. When this is to be done with DNA from an organism which normally does not exchange DNA with *E. coli*, we strongly suggest P3 level containment simply because of the 'built-in' unknown factors in such work.

(b) PURIFIED CELLULAR DNA'S OTHER THAN PLASMIDS, BACTERIOPHAGES, AND OTHER VIRUSES

"The formation of DNA recombinants from cellular DNAs that have been highly purified by physical and chemical techniques (i.e. not by cloning) and for which there is sufficient evidence that they do not contain harmful genes can be carried out under lower containment conditions then used for

the corresponding shotgun experiment." Basically, we agree with this statement from the Woods Hole Guidelines. But, we do feel that a better definition for and test of 'purity' must be assigned. Simple one-dimensional electrophoresis is likely to contain contaminating DNA molecules to the 1% level. Instead we would suggest two-dimensional gel electrophoresis is followed by cloning followed by repurification of the cloned material to test for original properties as a better test. In any case where purified but hazardous or obviously potentially hazardous DNAs are involved we would specify P4 containment plus strong justification and written approval by the recombinant DNA committee. We also maintain, that due to the 'unknowns' inherent in this work, all other such studies should for the foreseeable future be kept at the P3 level containment.

(c) PLASMIDS, BACTERIOPHAGES, & OTHER VIRUSES

(i) Animal Viruses

We very strongly feel that the experiments mentioned in this section of the Woods Hole Guidelines, those specifically dealing with animal virus DNA recombinants propagated in *E. coli* hosts, should be deferred until all of the following objections or questions are fully answered:

1. the selection, testing, and acceptance of real standards of 'purity' for the DNAs in question;
2. some real criterion for certain judgement that DNA's do not contain 'harmful' genes
3. that genome segments are absolutely free of latent or active genes involved in cellular transformation and oncogenesis; the division of tumor virus genomes into oncogenic and non-oncogenic sections, although reasonably convincing as it stands, is nonetheless an operational definition arising out of specific, limited experiments. Until the function in mammalian cells of all gene products from "non-oncogenic portions" of oncogenic viruses is known, putting them in *E. coli* should be deferred.
4. the epidemiological capacity and competence is shown to exist for the surveillance and discovery of any possible 'accident' with oncogenic or pathogenic viruses.

(ii) PLANT VIRUSES

From the above sections of this critique, our skepticism as to the existence of any proof or definition of DNA 'purity' should be as obvious as our strong objections to the use of an airborne and widely distributed host such as *E. coli*. We consider segments of a virus to be as potentially hazardous as the complete genome. It would therefore be prudent to propose P3 containment for all plant virus experiments with P4 being mandatory in the case of oncogenic plant associated plasmids or viruses.

(iii) EUKARYOTIC PLASMID DNAs

We feel that such experiments with DNA from warm-blooded eukaryotes should be deferred indefinitely for the reasons outlined for shotgun experiments on eukaryotic DNA recombinant molecules. For similar reasons we feel that plasmid DNAs from all other eukaryotes should be limited to P4 containment until safer host-vector systems are developed and documented.

(iv) PROKARYOTIC PLASMID & PHAGE DNAsPlasmid and Phage From Hosts that Naturally Exchange Genes with E. coli.

DNA recombinants formed from plasmid genomes or phage genomes from phages known to propagate in cells with known antibiotic resistance should only be studied under P3 containment. This also holds for experiments with recombinants from plasmid or phage genomes that have not been characterized with regard to pathogenic components. We consider P3 to be the minimal level of containment for any experiments not specifically mentioned here but of this category. Any experiments involving recombinant DNA of known pathogenicity or from pathogenic strains also mandates P3 containment. Experiments with known non-pathogens' plasmid and phage are P1-P2.

Plasmids and Phage from Hosts that do not Naturally Exchange Genes with E. coli.

Because of the previously described inherently high degree of hazard using an EK host system, all DNA recombinants formed with plasmids or phage containing resistance genes to clinically significant antibiotics should be deferred. Since no adequate and acceptable definition for "highly purified and characterized" segments of plasmids and phage genomes presently exists, all work with such putatively purified and characterized genome segments would wisely be done at P4 containment. Once it is demonstrated that such work is not potentially of high risk, these studies can then be dropped down to P3 containment.

Note: cDNAs (i.e. complementary DNAs) synthesized in vitro carry with them the analogous system of physical restraints.

(d) EXPERIMENTS WITH OTHER PROKARYOTIC HOST-VECTORS

The major thrust of our opinions on this section is presented earlier where we discuss the EK system and the essential problem of finding another and more suitable biological host system. Once this host-vector system is decided upon, fully studied, and then accepted, studies in other systems should be dropped from recombinant DNA analysis. Until this new host vector system is settled upon, all other non EK system studies can be deferred. The exception to this, of course would be specific strains being agreed upon as potential and safer replacements for the present EK system. And initially this work, depending on the studies being carried out, should be restricted to P3 or P4 containment.

B. Experiments with Eukaryotic host-vectors.

(a) ANIMAL HOST-VECTORS.

Only cells which have been extensively studied as tissue culture lines should be used as hosts. P3 containment levels are recommended with these hosts for work using mitochondrial DNA and that from low-risk viruses. Experiments with DNA from viruses which are detrimental to economically important animals and plants should be upgraded to the P4 level. Transference of the experimental system to the whole animal should be justified before a peer review committee. Experimental set-ups using highly virulent or oncogenic viruses should be deferred until our experience substantiates their usefulness over the risk involved.

The use of well characterised, modified viral genetic material as a highly contained vector for cloning in eukaryotic cells should be restricted to P4 containment facilities. We feel our caution is warranted because of the unknown hazards. A pathogenic, but low-risk virus has the potentiality to acquire greater virulence in a foreign environment with abnormal controls and targets on release. Logically, experiments with oncogenic viral DNA should be halted because of the greater risks involved, and the epidemiological difficulties of tracing causation of increases in incidences of specific cancers.

(b) PLANT HOST-VECTORS.

A word about the containment levels. P2 containment as described is minimal and appears a token gesture to standard microbiological technique. Because of the very nature of this type of work, up to now the techniques are much cruder than those used in microbiology. Therefore, we strongly recommend that cloning experiments in tissue culture be carried out at least at the P2 microbiological containment level when the plant is known not to harbour a pathogen. All other experiments with pathogen-containing tissue, detached plant parts and whole plants should be up graded to the P3 containment status.

In plants the modes of dispersal of pathogens do not necessarily depend on cell to cell contact either within the same plant or with other plants. Insects act as very efficient vectors of pathogenic agents, while spore-producing pathogens depend on either animals or air for their transmission. Thus, we feel that the most stringent conditions must prevail until further research and expertise with plants and plant tissue culture guarantees that accidental "spills" can be eliminated or proven non-hazardous.

Since it is still entirely unknown whether there will be rewards of so called "shotgun" experiments with whole plants, detached parts and cell culture, it is our opinion that such experiments should be halted at this time. Work with DNA which has been purified as rigorously as possible (see above) and then introduced into plant cells in culture would be a more easily controllable system in which to test whether such experiments are worthwhile.

(c) FUNGAL, or SIMILAR LOWER EUKARYOTIC HOST-VECTORS.

We strongly disagree with the sentiments of the first sentence of this section in the Woods Hole Guidelines - "The containment criteria for experiments on recombinant DNAs using these host vectors most closely resemble those for prokaryotes, rather than the proceeding eukaryotes, in

that the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria". Because of specialisation in the reproductive cycles the fungal spore is highly adapted for dispersal by air, water and animals. In addition, the organisms themselves are known to produce unusual substances - antibiotics, alkaloids and other highly toxic agents. Moreover, industrial antibiotic producing strains have been shown to contain large amounts of double stranded RNA virus. Thus, even at this speculative stage we urge that these organisms be used with greater stringency than prokaryotes.

Part III. IMPLEMENTATION

The evaluation and implementation of meaningful guidelines on artificial DNA recombinant experimentation face at least three very difficult obstacles:

- Evaluation and possible curtailment of experimentation already in progress.
- Evaluation of experimentation through a peer review process. Peer review is considered generally useful as a procedure for the distribution and awarding of research funds. On the other hand, the function of biohazard review groups will be to apply persuasive pressure or to withhold research funds due to violations of safety standards or improper containment levels for a particular research proposal. This is most likely going to be difficult for peers. Non-molecular biologists such as medical microbiologists, occupational health and safety epidemiologists, ecologists, and evolutionary biologists, as well as, informed representation from the public, would contribute to resolve difficult problems of interest that will, predictably, arise from a peer review process. A committee like the one we propose could follow the efforts of the National Science Foundation to involve the public in these kind of issues. The Provisions of the NSF Authorization Act, 1976, directs the Foundation to facilitate participation of citizens groups and scientists in the resolution of public policy issues involving scientific and technical aspects.

- Scant precedent exists in universities and other institutions for matters of safety to be decided in a pluralistic manner with the participation of all those who incur risks.

In addition, we do have precedents that point to the very real difficulties of organizing a molecular biological community around safety issues, especially on a voluntary basis. Dr. Andrew Lewis, for example, had an apparently disappointing experience in relying on voluntary compliance by investigators interested in studying the SV40-adenovirus hybrids that he isolated. Major labs did not support, or broke their support, or simply ignored a Memorandum of Understanding and Agreement to restrict distribution of these viral strains. Furthermore, the casual handling, in molecular biology labs, of reagents that are known to be carcinogens is already a source of concern in many quarters. The handling of organisms of yet unknown properties that have the capacity to multiply can only increase this concern in many of us. Finally, disappointing precedent comes from work involving entirely known biohazards. The number of reported acquired infections in laboratories with special containment facilities has been around 1650 in the last 30 years (Science, 187, 931, 1975).

In the light of the above considerations, we would like to see the following elements in a review system:

1. - Biohazard decisions should be a matter of public record including the arguments for and against the decision.
2. - Grant applications should include a Biohazard Impact Statement. This would describe not only local hazards to lab personnel but possible danger to the general public including possible long term effects. It would also serve as a means of self-education for the investigator. The impact Statements should be readily accessible to people in the lab to encourage discussion of safety issues.

3. - Establishment of procedures that will assure continued epidemiological monitoring of people (and their families) in places where artificial DNA recombination experiments are performed. The National Institute of Occupational Safety and Health (NIOSH), which under the Occupational Safety and Health Act (OSHA) of 1970 was charged to determine the potential dangers for hazardous chemicals in the workplace, appears to be the appropriate agency for this task.

The implementation of local Biological Safety Committees will need more support, considering that there is already a noticeable decrease in enthusiasm among the scientific community to carry out this part of the Provisional Statement of the Asilomar Conference. It is unfortunate, in our view, that the Final Asilomar Statement does not mention broad based safety committees. Instead, matters of safety, education and training are left to the responsibility of the principal investigator. This is not really satisfactory. The Safety Committee would have to be designed based on the principle that workers have a right to seek assurances and reasonable proof that their health is not at risk. A person must be offered an administrative mechanism by which she or he "can blow the whistle", without endangering his or her job status. This mechanism could involve the funding agency immediately looking into reports of violation of the guidelines. Advice from NIOSH on how to set up such a mechanism of grievance would seem most appropriate at this time.

Important and essential responsibilities of the local Safety Committee have been listed in the Current Guidelines of the Recombinant DNA Molecule Program Advisory Committee of the NIH. However, no mention is made as to the composition of the Safety Committee, nor to its possible role in the epidemiological monitoring

of the people exposed. Detailed points on epidemiological monitoring of lab personnel and their close contacts have already been offered in relation to surveillance of human cancers among personnel in virus labs (See Biohazards in Biological Research, pg. 309, Cold Spring Harbor Lab, 1973). The participation of independent expertise (including at least an occupational health and safety epidemiologist and a medical microbiologist) not directly involved in cloning experiments, is advisable. Participation of the people actually exposed to the health hazard including the maintenance and custodial personnel, seems eminently reasonable. Formal and educational and training sessions, as called for in the Provisional Asilomar Statement, should gradually allow informed participation of the non-experts in the laboratory.

THE DETRICK EXPERIENCE AS A GUIDE TO THE
PROBABLE EFFICACY OF P4 MICROBIOLOGICAL CONTAINMENT FACILITIES
FOR STUDIES ON MICROBIAL RECOMBINANT DNA MOLECULES*

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I. INTRODUCTION

Concern has been expressed for safety of the general public and for research personnel during research on recombinant DNA hybrid microorganisms (1). There appear to be assumptions that, first, a recombinant can, and may, be developed that will equal or exceed the virulence, infectivity and contagiousness of any presently known microorganism, and, second, that this agent will be transmitted to the general public either by infected laboratory personnel or by microorganisms leaving the laboratory on clothing, or in refuse, dead animals, sewage, or air.

There is no doubt that the hypothetical hybrid can be hazardous for all persons entering the laboratory, as is clearly evident from the many reports and analyses on laboratory-acquired infections. The value of containment facilities for laboratory-associated persons will be discussed in Section III.

The second assumption has almost no supporting epidemiological experience. Review of the literature yielded 35 accounts of laboratory microepidemics (Table 1). These can be examined for their effect upon the general public. Almost all of these epidemics occurred in the absence of safety equipment and of adequate efforts to control contaminated air, sewage, refuse, and laundry. Usually no more than rubber gloves and surgical masks were used during work on an open bench top. This is not unique. The history of microbiological research is characterized by a primary emphasis upon protecting the experiment rather than upon protecting the experimenter. Most work with human disease-producing microorganisms has been and is done on open bench tops with few or

*Research sponsored by the National Cancer Institute under Contract No. N01-CO-25423 with Litton Bionetics, Inc.

no precautions in the handling of air, sewage, refuse, laundry, or access to the laboratory. The ventilated safety cabinet, which is the principal safety device to protect the laboratory worker from exposures to infectious agents, was not commercially available until 1950. It also is significant that the National Institutes of Health did not have a research laboratory specifically designed to provide safe conditions for research with infectious agents until 1947 (2).

Nevertheless, it has not been possible to find reports of laboratory-attributed infection in persons who never were in the laboratory building or who were not associated in some way with the laboratory. Reports of infection by association include (1) Q fever from unsterilized laboratory clothing in six employees in a commercial laundry (3), (2) two persons involved in laundry, cleaning, and contact with a technician in a rooming house during a laboratory outbreak of Q fever (3), (4) Q fever in a switchboard operator in a nearby building - extent of contact with laboratory personnel is unknown (5), (4) Q fever in a wife whose husband was believed not to have taken the required shower before going home (6), (5) facial anthrax lesion in the wife of a bacteriologist during a time (January 1946) when there were several laboratory cutaneous infections (6,7), and (6) nine hospitalized and 26 non-hospitalized cases of Q fever in employees of a rendering plant that processed Q fever-infected guinea pig carcasses from a laboratory (8). No other laboratory-associated cases were found, although there are a few instances of a wife becoming infected after her husband contracted an occupational illness, and several infections of visitors.

The preponderance of Q fever reflects the unusual stability of the etiologic agent and the small amount required to infect man (1 to 10 micro-organisms), although the same low human infective dosage also applies to the

etiologic agents of tularemia, typhus, Venezuelan encephalitis, and others (9).

The absence of proved laboratory-initiated public hazard also is reflected in the Godber Report (10), which, from among all the agents listed in Table 1, places only Marburg virus in Category A, "Pathogens presenting hazards primarily or significantly to the human community."

The one exception to the above discussion is the recent instance in England of fatal smallpox transmitted to two visitors by a hospitalized vaccinated laboratory technician mildly ill with smallpox undiagnosed at the time of the visit (11). The Godber Report was a consequence of the two deaths. However, the infection of the two visitors is primarily an affair of medical practice and hospital epidemiology. For the purpose of evaluating the efficacy of P4 containment, the value of this unique episode is its indication that the only publically hazardous recombinants will be those that carry the genes that insure person-to-person transmission typically demonstrated by such diseases as smallpox, chicken pox, and measles. Without that gene (if there is such), the recombinant disease will be controlled by the usual hygienic and public health practices of our civilization. Furthermore, if person-to-person transmission is going to happen, early warning will be given by the appearance of carriers or illnesses in the laboratory population. This warning would not occur if all laboratory-associated personnel were effectively vaccinated against the contagious recombinant, which is a most unlikely situation. Examination of the report by Pike (12) on 3,921 laboratory infections shows that at least 95% were noncontagious diseases, and, if one excludes tuberculosis, less than 5% consisted of diphtheria, Neisserian meningitis, plague, syphilis, and viral respiratory (e.g., influenza), enteric (e.g., coxsackie), or exanthematous (e.g., vaccinia-smallpox) diseases.

The assembled reports conspicuously fail to include any accounts of transmission to the general public except as mentioned above. The 3,921 infections include 389 "overt" Detrick cases, according to a letter from the late Dr. S. E. Sulkin, dated 4 September 1969.

TABLE 1. EXAMPLES OF MULTIPLE LABORATORY INFECTIONS IN ONE INSTITUTION

Disease or Agent	Probable Source	Persons Infected	Reference	
			Ref.	Date
Brucellosis	7 U. S. Brucella research centers	66	13	1941
Brucellosis	Centrifuge accident	94	14	1940
Coccidioidomycosis	Culture transfer	13	15-16	1950
Coxsackie virus	Spill from tube	2	17	1950
Erysipeloid	Vet. students dissecting horse	13	18	1940
Hepatitis, viral, B	Cuts from computer cards	5	19	1974
Histoplasmosis	Routine lab work	9 overt	20	1956
Histoplasmosis	Med. Tech. student lab.	18a/	21	1955
Histoplasmosis	Microbiol. student lab.	26b/	22	1964
Leptospirosis (ballum)	Handled infected mice	8	23	1950
Louping ill virus	Intranasal inoculation of mice	3	24	1934
Lymphocytic choriomeningitis	"Infected hamsters or fomites"	10	25	1966
Lymphocytic choriomeningitis	Silent infection in hamsters	48	26	1975
Lymphocytic choriomeningitis	Silent infected mice to 3 mouse and 3 hamster colonies in 7 labs.	23	27	1973
Marburg virus	Monkey blood and organs	23	28	1968
Psittacosis	"May be parrot 'powderdown'"	11	29	1930
Q fever	"Probably due to dust"	13	30	1949
Q fever	"Rising (animal) dust"	15	31	1940
Q fever	Use of a centrifuge	15	32	1947
Q fever	Harvesting infected eggs	16	33	1946
Q fever	Harvesting infected eggs	20	34	1946
Q fever	Not stated (German lab.)	20	30	1949
Q fever	Use of a centrifuge	47	35	1947
Q fever	Use of a centrifuge	60	36	1946
Q fever	Watch autopsy sheep (students)	150	37	1964
Rift Valley fever	Lab., and animal handling	11	38	1949
Tularemia (RMSF Lab.)	Unknown-routine lab. work	6	39	1926
Tularemia (NIH)	Handling, dissecting rodents	6	40	1921
Typhus fever	Unknown (NIH lab.)	17	41	1944
Typhus, epidemic	Making Weigl's vaccine	17	42	1940
Typhus, murine	Intranasal inoculation of mice	6	43	1942
Typhus, murine	Unsterilized glassware	7	44	1954
Typhus, murine	Intranasal inoculation of mice	12	45	1943
Venezuelan equine encephalitis	Drop, break, lyophile tubes	24	46	1959
Vesicular stomatitis	Lab., and animal handling	54	47	1958
Viral hemorrhagic fever	Airborne dry rodent urine/feces	113	48	1962
Yaba-like disease	Handling monkeys	5 & 15	49	1967
			50	

a/ 2 overt cases; b/ 1 hospitalized; 25 mild or subclinical infections

II. DEFINITIONS

In discussions about precautionary facilities desirable to minimize human risk accompanying research with recombinant DNA hybrid microorganisms, it is not uncommon to hear the Fort Detrick experience quoted as proof that infection of laboratory personnel cannot be prevented. It is true that good engineering design is not a completely adequate substitute for thoughtfully applied good technique, but the reverse also is true. Both are necessary.

A typical public statement is as follows: "Even in the P4 conditions of the Army's biological warfare laboratories at Fort Detrick, there were 423 cases of infection and three deaths over some 25 years" (1).

Detailed analysis of the conditions under which those 423 infections were acquired does not support the statement, because P4 conditions, insofar as this requires biological safety cabinets or a gastight cabinet system with attached gloves, never was present in all the laboratories during the 25 years' experience. P4 and P3 conditions are defined as follows (51):

"P4 level (high). Work areas are in a special facility of the type designed to contain highly infectious and hazardous microbiological agents. These areas are isolated by airlocks, a negative pressure environment, personnel clothing change and shower rooms, and treatment systems to inactivate or remove biohazardous agents contaminating exhaust air, liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the facility. The handling of the biohazardous agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through HEPA filters."

"P3 level (moderate)". In addition to the P2 procedures, this level requires the following: (i) Operations with potentially hazardous organisms that require P3 containment should be carried out in a laboratory that is separated from areas where less than P3 containment is practiced. The ventilation system shall be balanced to provide for an inflow of supply air from the adjacent corridor into the laboratory through the access doorway. This supply airflow shall be in excess of 50 cubic feet per minute. The general exhaust air from these laboratories should be discharged outdoors and dispersed to the atmosphere to prevent reentry into the building. If recirculated, the air must be decontaminated. Appropriate biohazard signs should be posted at access points to the laboratory, and only persons specifically authorized by the principal investigator should enter when these signs are posted. Normally, authorized persons should be limited to those who work in the laboratory. (ii) Properly installed biological safety cabinets, meeting appropriate NIH performance standards, should be used for all transfer operations and for all procedures likely to produce aerosols. Operations that produce large amounts of aerosols, such as blending, sonication, vortex mixing, and vigorous agitation, should be avoided where possible. Where necessary, they must be carried out in enclosed systems that are not opened until adequate time has been allowed for aerosols to settle. (iii) Gloves should be worn during the handling of biohazardous materials. (iv) Vacuum lines should be protected by filters.

P3 level research can be conducted in laboratories where the room airflow and general exhaust air conditions described above cannot be achieved, provided that this work is contained in closed systems which are maintained under a negative pressure of at least 0.25 inches water gauge. Operations including centrifugation, fractionation, sonication, etc., which involve the

handling of recombinant DNA molecules shall be contained in these systems. All materials, before removal from these systems, shall be sterilized or transferred to a sealed unbreakable container which is then removed from the system through a chemical decontamination tank, or after the entire system has been decontaminated."

P3 "properly installed biological safety cabinets, meeting appropriate NIH performance standards," for the purposes of the comparative evaluations in this report, are approximately equal to cabinets referred to in the literature, safety manuals, and manufacturers' advertising, as (1) Class I cabinet (inward airflow of 60 to 100 linear feet per minute through a variable or fixed (8 to 10 inch) cabinet-wide opening for the hands; (2) Class II cabinet (partially recirculated sterile air in a downflow cabinet with an inward airflow and fixed or variable opening as in the Class I); (3) Biosafety cabinet; (4) Biological safety cabinet; (5) Microbiological safety cabinet; (6) Biohazard safety cabinet; (7) Open face safety cabinet; or (8) Biogard hood. In all these, the exhaust air passes through a microbiological filter.

Negative pressure environment. This is a design in which the air flows from outside the building, into the "clean" area, if any, into the laboratory corridors, and into the laboratory rooms, from which the air is exhausted. In some special situations, there also is an exhaust in airlocks, but usually not.

At this point, because of the definition of P3 and P4, it seems pertinent to comment on the value of secondary barriers. Secondary barriers become especially important to personnel within the building when such agents as Coxiella burneti, experimental aerosols, or micronized dry powders are handled, or when there are centrifuging, grinding, or similar aerosol-producing

procedures without containment precautions, accidents with lyophilized tubes, animals excreting large amounts of infectious bacteria or virus, or pilot plant operations.

In some laboratories, the secondary barriers contribute significantly to integrity of the experiment by reducing nonspecific or cross-infection of materials or animals.

As far as biohazard outside the building is concerned, most secondary barriers are more for reasons of public relations than for anything else, except for pilot plants or other large-volume production, experimental aerosols, use of tick or insect vectors, and agents capable of spread to the animal or plant food supply. This view assumes that known infectious liquids, solids, animals, and animal wastes are decontaminated before disposal, as has long been standard practice in all microbiological laboratories.

It is difficult to determine the importance of negative air balance within a building. In addition to those microepidemics in Table 1 in which the presence of a negatively balanced air-handling system might have reduced the number of infections, there are at least two episodes in which a defective air balance was the determining factor: (1) transmission of hog cholera in the large animal isolation facility of the National Animal Disease Laboratory at Ames, Iowa (52), (2) an unpublished account of a case of psittacosis in Building 539 at Fort Detrick in 1961, in an unvaccinated new technician who had never worked with this agent but who worked in the fourth laboratory room down the corridor from a room in which a Henderson aerosol exposure apparatus (54) was employed for the head-only aerosol challenge of mice and guinea pigs. The day of probable exposures was suitable to explain the day of onset of illness. It must be admitted that maintaining an uninterrupted flow of air from the corridor to all laboratory rooms is virtually impossible, because

the door to each laboratory room sometimes is left open and sometimes is kept closed, and because the airflow into ventilated cabinets may be intermittent. The latter condition has caused some installations to operate the blowers of ventilated cabinets continuously as part of the building ventilation system. Of course, the air balance then requires adjustment whenever a ventilated cabinet is added or removed.

III. THE DETRICK EXPERIENCE

Fort Detrick was activated on 10 April 1943, and an operational safety officer for the pilot plant was appointed within the first month. By September 1943 a strong safety organization was organized and staffed principally by physicians and bacteriologists, most of whom were in military service. Formal written safety regulations were issued and well received by the laboratory personnel. However, the protective cabinets characteristic of P3 and P4 containment systems were absent. The years 1944 through 1969 can be divided into three time periods, based upon the gradually increasing use of Class I ventilated cabinets and gastight Class III systems, and the development of effective vaccines for anthrax, tularemia, and Venezuelan equine encephalitis. No infections were reported in 1943.

1. 1944-50 inclusive, with 147 infections

Facilities. During this time, there were no major primary barriers such as ventilated cabinets and ventilated closed cages to supplement the usual laboratory benches, except in one building, which was devoted to the study of microbial aerosols and the aerosol challenge of animals. This Building 524 and its activities have been described in detail by Rosebury (53). The building had no protective ventilated cabinets, but the more

hazardous operations preparatory to aerosol challenge were done in a Reyniers germ-free chamber with arm length attached gloves. Animals were kept in ventilated closed cages with exhaust air passed to an oil-fired incinerator. Secondary barriers were present in a developmental stage in Building 524 and in most of the other laboratory buildings (restricted access; air locks; exit autoclaves; refuse incineration; exhaust air treatment that provided a negative air balance in the building; clothing change; exit showers).

Infections, 1944-50 inclusive. There was a marked difference between the number of infections in Building 524 in which hazardous experiments were done with various infectious microbial aerosols inside containment equipment, and the number of infections in other buildings of reasonably comparable size in which basic research confined to a single microbial species was done on open bench tops. No experimental aerosols were generated in these other buildings.

In Building 524, in the 6 months 21 March 1945 to shortly after the end of World War II, with intensive study of aerosols of P. tularensis, B. suis, M. mallei, M. pseudomallei, and psittacosis agent, there was only one infection, caused by a leaking frozen ampule of psittacosis agent handled on the open bench top. There were no ventilated cabinets in use at Fort Detrick at that time. The absence of ventilated safety cabinets to use in preparing test materials and in making examinations subsequent to aerosolizations in the gastight steel chamber was a major safety weakness. Operations ceased late in 1945 and were resumed sometime in 1946. The subsequent record of only 5 cases of brucellosis in Building 524 during 1946-50 also was good, considering the obvious hazard associated with aerosol clouds in the chamber and adherence of the test microorganisms to the hair of experimental animals, mostly mice and hamsters.

1944 to 1950, inclusive

Bldg. 524: Brucellosis - 5 cases; psittacosis - 1 case

Bldg. 429: Brucellosis - 22 cases

Bldg. 432: Brucellosis - 15 cases

Bldg. 504: Tularemia - 9 cases in 1944-46

Bldg. 525: Tularemia - 26 cases

Other buildings and the pilot plant accounted for the remainder of the 147 infections.

2. 1951 - 1959, inclusive, with 224 cases (99 were tularemia)

In 1950, the first experimental ventilated protective Class I cabinet was tested and the first commercial stainless steel models were delivered by S. Blickman Company. The first cabinets were placed in buildings in which only brucella was under study, because of the severity of the disease and the absence of effective antibiotic treatment. The "tularemia buildings" were the last to receive protective cabinets, because use of a killed vaccine modified the severity of the disease and antibiotic treatment was effective. By 1954, there were 11 buildings containing at least 87 of the new stainless steel ventilated cabinets, mostly of the size 60 inches wide; none were of the 36 inch size. Another 7 buildings had 13 of these safety cabinets.

It is difficult to select examples of P3 and P4 containment during 1951 through 1959, because this was a period of transition from old to new buildings and realignment of research. Also, certain buildings must be excluded because their activities were remote from anything likely to be done in recombinant DNA research. The excluded buildings were those in which experimental aerosols were studied as such or by animal challenge, in large tanks or in the 1,000,000 liter armor-plated sphere, and the pilot plants and associated laboratories engaged in large-scale production of microorganisms or toxin.

Building 376, with Class I and Class III Cabinets. Nevertheless, Building 376, specializing in study of lesser-volume aerosols from liquid

or micronized microbial dry powder, and aerosol challenge of animals from mice to monkeys, is a demonstration that a combination of Class I cabinets and Class III gastight cabinets, together with all the usual secondary barriers, can reduce the number of, but cannot prevent, laboratory infections when highly infective organisms are handled as aerosols. This modern brick building, with 38,600 square feet of space, was completed in May, 1953. It contained 14 ventilated Class I steel cabinets 60 inches wide, and 5 steel 72-inch wide animal-transfer cabinets to use in conjunction with ventilated steel animal cages. There were more than 200 linear feet of Class III gastight cabinets, a pneumatic tube system to convey small animals from aerosol exposure chambers, of the Reyniers type, to the animal caging rooms, and a complex optical system to bring in sunlight to study the effect of its various wave lengths upon microbial aerosols.

Infections were as follows: 1953 - 1 brucellosis; 1954 - 3 tularemia, 2 Q fever; 1955 - 2 Q fever, 2 Venezuelan encephalitis (VEE); 1956 - 2 tularemia; 1957 - 1 brucellosis, 1 VEE; 1958 - 3 tularemia, 1 cutaneous coccidioidomycosis from an accidental syringe inoculation; 1959-68 - no infections; 1969 - 2 nonhospitalized tularemia cases.

An effective cell-free anthrax vaccine was introduced in 1952 and extended to craftsmen in July 1958. The introduction of live tularemia vaccine in June 1959, and live VEE vaccine in 1963 ended infection with those agents.

The effectiveness of containment systems in Building 376 can be better appreciated with knowledge of the numbers of animals challenged with microbial aerosols, the etiologic agents under experimentation, and a realization of the amount of laboratory work preceding and following the aerosolizations.

For instance, in 1959 there was aerosol challenge of 46,412 mice, 3,013 guinea pigs, 25 rabbits, 276 monkeys, and 2 chimpanzees. Work included the etiologic agents of anthrax, coccidioidomycosis, plague, psittacosis, Q fever, Rocky Mountain spotted fever, tularemia, Venezuelan encephalitis, yellow fever, and two others.

3. 1960 - 1969, inclusive, with 52 infections

These 10 years featured the introduction of an effective live tularemia vaccine in June 1959, with the result that there was only one hospitalized case, in 1961 in a man who had not received the living vaccine, although he had a long history of 17 injections of killed vaccine. There were 10 mild nonhospitalized tularemic infections; 9 had had the live vaccine and 1 with a cutaneous lesion had had pulmonic tularemia in 1957. There was 1 hospitalized VEE case in 1961. But after vaccination with live VEE vaccine began in 1963 there were only 3 mild cases. These three had had the live vaccine.

There were a few cases of other laboratory infections, but the only single agent causing a significant number was Coxiella burneti (Q fever); there were 22 cases, of which only 7 were ill enough to require hospitalization. The 22 infections were scattered among 8 buildings, not including 3 infections of uncertain origin among craftsmen and engineers.

From the viewpoint of evaluating effectiveness of Class I cabinets, the record of Building 560 is noteworthy. This building housed medical microbiological units responsible for basic research on (1) tularemia, (2) plague, (3) genetics, (4) bacterial nutrition, (5) microphysiology, and (6) staphylococcal toxin.

There were over 100 persons daily at risk in the six units, but most of the microbial agents were not highly infectious. Tularemia infections

were absent because of protective vaccination. P. pestis is a fragile organism requiring a considerable respiratory dose, as is evident from Pike's report (12) of only 4 laboratory cases in the United States and 6 worldwide. The Genetics Branch worked only with nonpathogens, although there was one hospitalized case of Salmonellosis, ascribed to mouth pipetting. The Bacterial Nutrition and Microphysiology Branches examined a variety of bacteria using Class I cabinets. During the 10 years there was only one infection, in 1963 - severe pulmonary coccidioidomycosis with a cavity in the lung. This was the consequence of using a shaker with a dry spore sediment and glass beads, inside a Class I cabinet with an inserted four porthole panel without attached arm length gloves. The staph enterotoxin unit had 4 illnesses, ascribable to a procedure most unlikely to be duplicated in any studies with recombinant DNA. This procedure involved exposing the heads of monkeys to an aerosol of solubilized aerosolized staph enterotoxin, using a Henderson apparatus in a modified Class I cabinet (54). No respiratory protection was worn during transportation of the monkeys (the fur on their heads obviously was shedding enterotoxin) to the animal room, where they were held in open cages.

Nevertheless, a summary made in June 1966 concerning the biohazards in the study of staph enterotoxin does show that Class I hoods were protective. Portions of the summary are reproduced below, from a contractor's report:

"Toxic reactions of personnel engaged in purification studies of the enterotoxin on an open laboratory bench have included mild cold symptoms, conjunctivitis, and dermatitis. The latter has been severe when concentrated material was contacted by the skin. A tolerance is developed after repeated exposure; however, skin reactions to the concentrated material continue."

"Of 15 persons engaged in laboratory purification studies of enterotoxin, one had conjunctivitis, one had a nondescript chemical irritation of the right eye, one had general skin reactions, one had a severe facial skin reaction, one had dermatitis, one had dermatitis and cold symptoms, one has observed no effects, and five were not reported on. No enteric reactions have been noted.

"Workers using laboratory hoods did not experience these reactions.

"One case was reported as having resulted from accidental ingestion of material during a filter cleaning process. The operator wore all prescribed safety equipment including mask, gloves, etc. Accidental ingestion was considered to have occurred during a smoking period, which involved either violation of regulations or insufficient preparatory decontamination.

"Conclusion: Oral pipetting, eating, smoking must be prohibited.

"Twenty-three cases were reported during an operation in large, open areas where personnel wore surgical masks or face shields. Most of the cases involved conjunctivitis and acute pharyngitis, and two cases of diarrhea and vomiting occurred. It appeared that even the seemingly innocuous gesture of brushing some exposed body part against clothing worn during processing was sufficient to initiate sensitivity reactions.

"Conclusion: Face shields and conventional surgical masks are not adequate."

4. Special Examples of Containment

P3. The best example at Fort Detrick, to demonstrate the probable effectiveness of a P3 system in recombinant DNA research that involves a

highly infectious agent, lies in the experience of the principal microbiological unit charged with basic research on tularemia, in Building 525. These personnel were moved in 1957 into one wing of a modern brick building, Number 560, completed in 1956 and equipped with all secondary barriers and with 12 Class I ventilated safety cabinets, then known as "Blickmans." About 65 linear feet of a gastight Class III cabinet system were installed several years later, but never used.

<u>Phases</u>	<u>Years</u>	<u>Primary Barriers*</u>	<u>Tularemia Infections</u>
1944-1953 Bldg. 525	10	All work on open bench tops. Secondary barriers were adequate.	35**
1954-1956 Bldg. 525	3	Six 6-foot modern Class I cabinets had been installed.	8
1957-1959 Bldg. 560	3	Twelve Class I cabinets	6
1960-1969 Bldg. 560	10	Live tularemia vaccine administered to all personnel.	None

* Also, centrifuge safety cups; leak-proof blenders.

** There were 6 Salmonella infections in a small group assigned to a Salmonellosis project, temporarily housed in Building 525 during 1951-1952.

P4. Demonstration of the effectiveness of a P4 system in which no Class I or Class II cabinets were used, and in which all work was done within gastight cabinets, can be seen from the record of an organization housed in Building 459 and later in Building 1412 (72,000 sq. ft.). All the usual secondary barriers were present.

1. Building 459, a concrete block, one-floor unit of 10,160 sq. ft., was completed in May 1945 and used for storage. In 1951, it was furnished

with gastight cabinets only, and assigned work of major hazard with the most infectious and toxic agents. This was basic and applied research using a variety of techniques, procedures, equipment, and small animals, with some incidental aerosol challenge of animals. At that time, it was the only organization with such equipment. The record is as follows:

1952:	One cutaneous chronic brucellosis
1953:	One nonhospitalized subclinical coccidioidomycosis, and one glanders
1954:	One nonhospitalized subclinical coccidioidomycosis
1955:	One tularemia
1957:	One hospitalized coccidioidomycosis
1958:	One tularemia
1959:	This organization moved into a new all-Class III Building 1412.
1959-1969:	One nonhospitalized cutaneous blastomycosis in 1966

2. Another separately administered research unit occupied that portion of Building 1412 designed for animal aerosol exposure in two stainless steel Freon-tight chambers of 18,000 cubic feet and 30,000 cubic feet, and in two smaller tanks each of 1,500 cubic feet volume. From December 1959 to December 1969, the 55 employees, of whom 45 were daily at risk, carried a weekly work load that commonly included the intracerebral inoculation of 6,000 to 8,000 mice, and the whole-body exposure to microbial aerosols of 200 to 300 guinea pigs and 20 to 30 monkeys with all the associated preliminary and subsequent procedures. Work was with such agents as those causing tularemia, plague, brucellosis, anthrax, Venezuelan encephalitis, coccidioidomycosis, botulism, and Q fever. There was only one infection (cutaneous, nonhospitalized) during the ten years.

DISCUSSION

1. In considering the 423 infections at Fort Detrick, in comparison to those reported elsewhere, it should be recognized that the occupational health program was unusually thorough in its active search for occupationally-acquired infections. The maintenance of large biological safety and medical departments also was characteristic of the Army biological operations at Dugway Proving Ground, Utah, and Pine Bluff Arsenal, Arkansas. Two unusual formally announced policies contributed to maximizing detection of occupational infection. The first was a policy that "every illness suffered by an occupationally exposed employee will be considered occupational until the medical service of Fort Detrick determines otherwise." The second was that "employees hospitalized by Fort Detrick physicians for illness suspected or known to have been caused by a BW agent under study will be carried on a 'duty' status without charge to annual leave or to sick leave." (This was justified by considering the patient as being a contributor to medical knowledge of a potential BW agent.) Of course, all such medical care was free of charge to the employee.

2. There is some difficulty in comparing the efficacy of the Detrick primary and secondary barriers with the probable efficacy of the proposed P3 and P4 barriers because the definitions of P3 and P4 quoted herein are preliminary and subject to revision. At present, neither P3 nor P4 requires use of "Class III" gastight cabinet systems, although the wording of P4 does not exclude Class III.

3. For the purposes of the following Conclusions, the Class I ventilated safety cabinet used at Fort Detrick is considered to be equivalent to the ventilated safety cabinets specified in P3 and P4 conditions.

IV. CONCLUSIONS

1. Class III gastight microbiological cabinet systems, which are neither required nor excluded by the definition of P4, can be operated without infection of the operators, in the very hazardous whole-body and head-only aerosol exposure of small animals and monkeys to USPHS Communicable Disease Center Class 3 and 4 etiologic agents, under standardized somewhat regularly repetitive procedures, with stable, well-trained, well-disciplined personnel (Detrick Bldg. 1412, Aerobiology Unit, December 1959 - December 1969).

2. When gastight cabinet systems are used for basic and applied research with Class 3 and 4 agents, in a changing variety of techniques, procedures, equipment, and experiments, there will be some laboratory infections, usually due to breaks in the attached rubber gloves, human exposure during entrance and exit of materials, leaks in the system, and human error. (Bldgs. 459-1412, during 1952 through 1969: Less than one infection every two years)

3. When a gastight cabinet system and Class I cabinets are both used in aerosol studies with Class 3 and 4 agents, there will be an increased number of infections, in comparison to use of only a Class III system. (Bldg. 376 - 18 infections during 1953 through 1958, and only 2 thereafter, mostly due to the introduction of live tularemia vaccine in 1959 and live VEE vaccine in 1963, which ended infection with those two agents. In the entire period 1953 through 1969, there were 20 cases in 17 years, which is a good record.)

4. A research program consisting principally of standardized repetitive procedures is less hazardous than a program that requires comparatively frequent changes in technique and equipment.

5. In the absence of effective vaccination, it is not possible to do basic research with a highly infective agent on the open bench top, nor with Class I cabinets that are accompanied by all the secondary barriers specified in P4, without laboratory infections. (Record during 1944-1950 and the record with tularemia in Buildings 525-560 during 1944-1969)

6. If neither P3 nor P4 requires use of Class III gastight cabinet systems, it will not be possible to prevent laboratory infections if an agent is used that is as infective as Pasteurella tularensis or Coxiella burneti. A definition of P4 that consists of adding secondary barriers (air locks, negative pressure environment, showers and change rooms) to P3 conditions will not result in a significant decrease in the number of clinical or sub-clinical laboratory-acquired infections. The addition will reduce the chance of infection of unauthorized visitors, personnel in rooms close to the recombinant DNA research unit, and a wife at home.

7. For research on recombinant DNA, the most effective safety measure to prevent infection of laboratory personnel is to utilize a microorganism that will not infect humans. Otherwise, the greater the required human infective dose, the safer it is.

8. Microorganisms for which there is an effective vaccine, and also in some cases specific effective therapy, could be considered for use in research on recombinant DNA.

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SUPPLEMENT TO 20 JANUARY 1976 REPORT ON
THE DETRICK EXPERIENCE AS A GUIDE TO THE
PROBABLE EFFICACY OF P4 MICROBIOLOGICAL CONTAINMENT FACILITIES
FOR STUDIES ON MICROBIAL RECOMBINANT DNA MOLECULES*

A. G. WEDUM, M.D.

29 January 1976

- I. Table I (Supplement). Examples of Multiple Laboratory Infections in One Institution
- II. Wives Infected by Husbands Employed in Microbiological Laboratories
- III. Experiments in Man with Oral Doses of Escherichia coli Hybrids

28 January 1976

Laboratory-Acquired Infections at the
Center for Disease Control (USPHS), Atlanta, Ga.

According to Dr. John Richardson, Chief,
Office of Biological Safety at CDC,
speaking informally at FCRC, Bldg. 550
on 28 January 1976, he may be quoted
as follows:

There have been to date at CDC
some 150-odd laboratory-acquired cases
(bacterial, viral, mycobacterial, fungal).
For 109 of these there is definite information
on probable causation, serology, virology,
laboratory epidemiology. There were no
secondary cases of transmission outside the
laboratory, except for one possible Shigellosis
(Boydii or Flexneri) in the child of an
infected female technician. The child had a
diarrhea at a time epidemiologically
compatible with transmission from the mother.
But the identity of the organism and its
correspondence to the agent causing the
mother's lab-acquired infection was
not established.

W. H. D.
28 Jan 1976.

I. TABLE 1 (SUPPLEMENT) EXAMPLES OF MULTIPLE
LABORATORY INFECTIONS IN ONE INSTITUTION

The use of Escherichia coli in the study of recombinant DNA molecules has caused concern that infection of laboratory personnel by a pathogenic hybrid might result in person-to-person transmission that would endanger the health of the surrounding community. In evaluating the extent of this possible hazard, it is useful to examine the extent to which laboratory-acquired intestinal disease has caused secondary cases. Table 1 (Supplement) identifies two small microepidemics of 4 and 6 cases and 4 surveys by German authors that included, with possibly a few minor exceptions, presumably only European laboratories. There were 265 cases. An important aspect of each survey, as far as the present discussion is concerned, is that the survey questionnaire included a query as to whether there were secondary cases. There were none, except as noted in Table 1 (Supplement).

TABLE 1 (SUPPLEMENT)
EXAMPLES OF MULTIPLE LABORATORY INFECTIONS IN ONE INSTITUTION

Disease or Agent	Probable Source	Persons Infected	Reference Ref. Date
Shigellosis	Presumably mouth pipetting	6	16 1954
Typhoid fever	"No direct accident"	4	10 1947
Typhoid fever	*Survey of European labs.	50	7a 1915
Typhoid; Paratyphoid; Shigellosis	*13-year survey of European labs.	75	7b 1929
Typhoid; Paratyphoid	*Survey of European labs.	70	8 1939
Typhoid; Salmonellosis	*Survey of laboratories	70	9 1950

*The survey included a question as to whether other persons were infected.
Replies: Typhoid - (1) Patient's nurse (Ref.7a); (2) Patient's residential
roommate (Ref. 8); (3) "Three other people infected themselves or were
infected either by the woman or directly by other means" (Ref. 7a).
(4) Patient's nurse (Ref. 9).
Cholera - (1) Wife of the patient (Ref. 7b)

II. WIVES INFECTED BY HUSBANDS EMPLOYED IN MICROBIOLOGICAL LABORATORIES

Cholera: During a severe and fatal infection of her husband, the wife washed his clothes at home, including bed clothes. After a severe diarrhea, she also died five days later (7b).

Bolivian hemorrhagic fever (Machupo): Wife, after visiting her husband in the hospital. "Direct person-to-person spread has not been reported in any of the other hemorrhagic fevers." (20)

Marburg disease: Wife, by sexual intercourse with her husband, 11 weeks after his recovery from a laboratory-acquired infection (21).

There have been no other cases in family contacts (22).

Anthrax: Cutaneous facial lesion in a wife who never visited the laboratory in which her husband worked, during a time when there were many cutaneous cases. Her husband was not infected. Case 24 (23).

Psittacosis: Wife, secretary in a building separated from the laboratory in which her husband was employed, hospitalized on the fifth day after her husband was released from the hospital after a ten-day illness with psittacosis (24).

Q fever: In a wife whose husband was believed not to have taken the required shower before going home (24,25).

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III. EXPERIMENTS IN MAN WITH ORAL DOSES OF ESCHERICHIA COLI HYBRIDS

Escherichia coli is especially useful in the study of hybrids because it can be changed in a great many ways by genetic transfer of plasmid-specified information from other microorganisms (391). The K-12 strain commonly used in recombinant studies was reported (463) as not surviving in the human intestines of nine volunteers for more than a few days. Two K-12 hybrid experimental vaccines in oral doses of 10^6 to 10^8 cells and 5×10^{10} cells have been tested safely in 45 and 57 men, respectively (26). A noninvasive toxigenic K-12 hybrid in oral doses of 10^6 to 10^{11} organisms was well tolerated by 85 of 86 men (538). Fortunately, man-to-man transfer of E. coli for all practical purposes is entirely oral. To change this basic characteristic may be difficult. The reported human infective dose of intestinal microorganisms is high (Tables 9 and 10) (539), although the low ID of S. Flexneri 2a (Table 9), and a report that 10 S. shigae were infective for 1 of 10 men (538), warns that the ID might be lowered if deliberately sought for by genetic manipulation. Although in selected instances extensive biohazard precautions would be necessary for research with E. coli hybrids, it is probable that the most important of all precautions would be the hardest to enforce, namely (1) use mechanical pipettors, (2) avoid hand contamination, (3) keep hands out of the mouth and off the face, (4) no smoking in the laboratory, (5) no candy, food, drinks, or gum in the laboratory. In this connection, the consensus of an international meeting at Asilomar in February 1975 (464) included a recommendation for the precautionary utilization of only those organisms unable to survive in the human intestines or outside of the laboratory. In December 1975 additional guidelines were developed (536).

In concluding this analysis of the potential biohazard of hybrid viruses, it is apparent that, although transmission to family and public is only a remote possibility, protective measures should be taken in the laboratory in anticipation of possible unexpected development of disease potential absent in the parental strains. For example, the temperature-sensitive mutant of measles virus can induce, in the newborn hamster, an hydrocephalus that is not produced by the parental strain or by other measles virus mutants (448).

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TABLE 9. INFECTIVE DOSE FOR 20-50% OF VOLUNTEERS

Disease or Agent	Inoculation	Dose
Scrub typhus (21)*	Intradermal	1/4 to 3 mouse ID50
Tularemia (22)	Intradermal	10-50
Q. Tularemia (22,23)	Inhalation	10-50
Malaria (24)	Intravenous	10
Syphilis (25)	Intradermal	57
S. Flexneri 2a (26)	Ingestion	180
~~S. dysenteriae 1 (Shiga) (538)~~	~~Ingestion~~	~~200~~
**Anthrax (27)	Inhalation	>1300 (greater than)
Typhoid (26)	Ingestion	10 ⁵
Tularemia (22)	Ingestion	10 ⁸
Cholera (28)	Ingestion	10 ⁸
Escherichia coli (29)	Ingestion	10 ⁸
Shigellosis (30)	Ingestion	10 ⁹

*Numbers in parentheses indicate references.

**1300 spores (500 less than 5 μ diameter) inhaled in 8 hours in a Pennsylvania and a New Hampshire Mill that processed contaminated goat hair. For many years employees had been inhaling 21 to 2200 viable B. anthracis particles daily. Yet at the Philadelphia plant during 1933-1 May 1958 there had never been a pulmonary case, but 119 cutaneous infections with one death. During 1956-57, 1/3 of the employees at this plant were vaccinated for the first time. South Carolina Plant: 23 (20 died) monkeys contracted pulmonary anthrax of 91 monkeys exposed to the exhaust air from a duct that vented a hood over the picking machine. This is a dramatic illustration of the value of the equivalent of a microbiological safety cabinet, which serves the same purpose of removing contaminated air, because among 250 employees there had been only 19 cutaneous cases during the 2-1/2 years the plant had been in operation. Another generality of interest to the cancer biohazard program, derived from these studies on anthrax, is that repeated comparatively small daily doses of anthrax organisms had no cumulative effect (156). From studies with 1,235 monkeys at Detrick, challenged with anthrax-bearing particles predominantly 5 μ or less in diameter, the LD50 was 4,130 spores, with 95% confidence limits of 1,980 to 8,630 spores. Apparently, for man or monkey, pulmonary anthrax is produced only when the minimum infective dose is given all at once.

Nevertheless, the absence of a cumulative human effect in anthrax may not apply to oncogenic viruses, judging from a study with the B/Tennant leukemia virus in BALB/c mice, in which it was found that "repeated inoculations with low doses of virus produce a significantly higher leukemia incidence than a single injection with a much higher dose" (408). This suggests that the moderate risk viruses should be examined by inoculation of susceptible animals with repeated small doses approximating the amounts that a technician might accidentally absorb.

Another aspect of variation in size of the human infectious dose depends upon individual differences in resistance to disease (Tables 10 and 11). This was demonstrated experimentally during studies on typhoid fever in man, in which 5% of the men resisted an oral challenge of 10^5 bacilli while the more susceptible 28% were made ill by ingesting 10^5 (253). Another example is available from the responses of chickens in the same inbred line highly susceptible to avian leukosis (188,215). Since humans are equally variable, then this gives impetus to the search for means to identify the constitutionally susceptible laboratory worker in viral oncology.

TABLE 10. HUMAN ORAL DOSE PRODUCING TYPHOID FEVER

<u>Dose of Viable <i>S. Typhosa</i></u>	<u>Percent Men with Typhoid</u>
10^3	None
10^5	28%
10^7	50%
10^9	95%

TABLE 11. AVIAN LEUKOSIS VIRUS, GAI STRAIN A

<u>Viral Particles Inoculated</u>	<u>Percent Chicks Infected</u>
10^4	5%
32×10^6	50%
10^{11}	95%



Letter to Recombinant Advisory Committee members

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

BETHESDA, MARYLAND 20014

March 19, 1976

Dear Dr. :

As you know, a special meeting of the Director's Advisory Committee was held at NIH on February 9-10 in order to review the guidelines proposed by your committee for DNA recombinant molecule research supported by the NIH. The meeting provided a forum for the scientific community and the public to comment on the draft guidelines. At that meeting, and subsequent to it, I have received a great deal of comment from a wide variety of sources. The comments generally have been most thoughtful and responsive. The participants at the meeting and those who wrote to me after it were unanimous in acknowledging the important contributions of your committee.

Your efforts in developing these guidelines are to be highly commended as exemplary of the constructive way in which the scientific community has risen to meet a singular challenge presented by its own achievements. The openness and candor that have characterized Asilomar proceedings, the self-imposed moratorium, and subsequent events have served both science and the public well.

It is apparent to me that the promise of recombinant DNA research is very great not only for scientific knowledge but also for its potential extensions to medical and other problems facing mankind. The guidelines the committee has proposed present a careful and thoughtful approach for the conduct of research involving DNA recombinant molecules. For the past month, I have been reviewing these guidelines with your chairman and other members of our staff in light of the comments and suggestions made by the participants at the meeting as well as the written correspondence thereafter.

As a result of that review, I have selected a number of issues that I ask you to consider at your next committee meeting. I would most appreciate your providing me with a rationale for the committee's having adopted a stated position in the guidelines as it relates to each of the issues raised by the commentators. Further, I ask in your response to the issue raised that you explain why you would accept or reject certain suggested changes in the guidelines. The issues are stated in the enclosed document, and are arranged according to the appropriate section of the guidelines. The document does not include some revisions and extensions being developed in the implementation section. It does include all substantive changes considered in all other portions of the proposed guidelines. Your responses to the issues I have selected will be taken into account when I make my decision within the month.

Thank you for your continued and invaluable contributions to this extraordinarily important matter.

Cordially,

/s/ Donald S. Fredrickson

Donald S. Fredrickson, M.D.
Director

Enclosure

March 19, 1976

SELECTED ISSUES FOR COMMITTEE REVIEW

A. Methods of Containment

Comments on the containment provisions in the proposed guidelines were directed to the definitions for physical and biological containment and the safety and effectiveness of the prescribed levels of containment. Several commentators found the concept of physical containment imprecise and too subject to the possibility for human errors. Others questioned the concept of biological containment in terms of its safety and purported effectiveness in averting potential hazards.

The commentators were divided on which method of containment would provide the most effective and safe system to avoid hazards. Several of the commentators suggested that there be a fuller explanation for each of the physical containment levels. Dr. W. Emmett Barkley convened a committee that has reviewed the section on physical containment in response to the suggestions. You will receive that committee's recommendations under separate cover.

Issues for the Committee's Consideration

There are approximately 27 P4 facilities in operation and 9 in construction. It has been suggested that the NIH should conduct a periodic assessment of these facilities when they are engaged in recombinant DNA experiments. The basis for this suggestion is that the nature of the experiments conducted at these facilities merits special attention by experts having maximum familiarity with the structure, operation, and potential problems of such facilities. What is the committee's response to this suggestion?

B. Prohibited Experiments

Commentators supported prohibition of experiments listed in the guidelines. Comments included suggestions for a clearer definition of the prohibition on experiments where antibiotic resistance may occur. It was urged by some that the prohibition be broadened to include experiments that result in resistance to any antibiotic irrespective of its use in medicine or agriculture. In this regard, I ask that the committee consider three separate sections of the guidelines that relate to the question of antibiotic resistance.

Issues for the Committee's Consideration

1. On p. 11 of the guidelines, new language is suggested to strengthen the wording of section (v) and leave no uncertainty with respect to the committee's intent in this regard: "Transfer of drug resistance traits to microorganisms that are not known to acquire them naturally should be deferred if they could compromise the present or future use of a drug to control agents that cause disease in any species of animals or plants."
2. On p. 28 of the guidelines, the following paragraph appears that relates to prokaryotes which do not exchange genetic information with E. coli: "Experiments extending the range of resistance to therapeutically useful drugs and disinfectants should use P2 + EK2 containment or higher depending on the virulence of the donor."

On p. 31 of the guidelines, the following statement appears concerning plasmids and phage from hosts that do not exchange genetic information with E. coli: "Experiments with DNA recombinants formed with foreign plasmids or phage containing genes which extend the range of resistance of the recipient to therapeutically useful drugs should use P3 physical containment + an EK2 host-vector."

These respective sections of the guidelines present certain problems that merit the committee's attention: First, there appears to be an internal discrepancy in stated containment levels in these latter two sections. Should not the containment levels for both be at P3 and EK2? The second problem, which is more serious in my view, is the relationship of these two sections to the prohibition as stated on p. 11. Was it the committee's intent to allow experiments where E. coli might be made resistant to therapeutically useful drugs (to which E. coli is not now known to be resistant)? If that is the committee's intent, then it would appear to be in conflict with the prohibitions stated on p. 11. I would appreciate the committee's opinions on this matter.

3. The guidelines state on p. 11, a prohibition of experiments using the DNA of pathogenic agents in Classes 3, 4, and 5 of the CDC Manual. Would the committee have any objection to broadening this to include not only their DNA, but also the DNA from any organism infected with these agents?

C. Biological Containment Criteria Using E. Coli K-12 Host-Vectors

The commentators were divided on the use of E. coli, strain K-12 as host-vectors. There were those who opposed the use of E. coli and others who strongly supported its use. I believe continuation of recombinant DNA work in E. coli is merited. I recognize as well the importance of NIH support towards developing alternative host-vector systems.

Issues for the Committee's Consideration

1. EK2 host-vectors: On p. 18 the first sentence cites "data from suitable tests performed in the laboratory." In response to comments made, would the committee expand on how one tests for survival of the cloned DNA fragment? Further, how would the committee view our incorporating the following changes in the text: " . . . This ~~absolute~~ measure of biological containment has been selected because it is a ~~realistic~~ measurable entity. Indeed, by testing the contributions of preexisting and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible ~~to-substantially indicate,-if-not-prove,~~ to estimate with considerable precision that the specially designed host-vector system can provide a margin of biological containment in excess of that required."
2. EK3 host-vectors: On p. 25 there is a one-paragraph description of this system. In light of possibly imminent certification of EK2 systems, would the committee consider defining the procedures to obtain independent confirmation of the specified containment?
3. The minutes of your December 4-5, 1975, meeting contain the statement that "the Committee passed a resolution that for the time being responsibility for certification of putative EK2 strains and publication of information on which it makes its certification lies with the Committee." However, no such statement appears in the guidelines. How does the committee view my inserting into the guidelines a statement that no host-vector is to be considered a bona fide EK2 or EK3 until it is certified by your committee?

D. Classification of Experiments Using the E. Coli K-12 Containment Systems

The commentators were clearly divided on the classification of containment criteria for different kinds of recombinant DNAs. Several commentators considered the guidelines to be too stringent and rigid. Others viewed the guidelines in certain instances as too permissive. And still others endorsed the guidelines as sensible and reasonable, affording the public an enormous degree of protection. Questions raised by commentators are those that have troubled your committee as well. The following issues are those to which I would like the committee's response.

Issues for the Committee's Consideration

1. On p. 26 of the guidelines for shotgun experiments with eukaryotic DNA recombinants, the committee has recommended lower containment levels for DNA from embryonic tissue. I concur with the committee's judgment that the likelihood of the presence of horizontally transmitted viruses is less in embryonic material than in non-embryonic material in the same species. But it is clear that careless handling of embryonic tissue can cause it to become infected. What is the committee's response to a stipulation in the guidelines that all such embryonic material be handled under aseptic conditions?
2. On p. 26 of the guidelines there are reduced containment levels for foreign DNA from cold-blooded vertebrates. I know the effort of the committee to resolve the difficult issues in this area. In my view, there is not available to us the arguments or evidence that support lower physical and biological containment levels for DNA from lower species. Unless there are compelling arguments to the contrary, I am considering raising the containment levels for cold-blooded vertebrates from P2 + EK2 to P3 + EK2 and, further, setting the containment levels for embryonic or germ-line DNA from cold-blooded vertebrates at P3 + EK1 or P2 + EK2 instead of the presently recommended containment level of P2 + EK1. What is the committee's response to this suggestion?

3. Another concern relates to the specified containment levels for "other cold-blooded animals and lower eukaryotes" on p. 26 of the guidelines. Of special concern is the frequency with which insects, for example, are known to carry agents pathogenic to man. The great uncertainty surrounding the use of DNA from these sources requires a conservative approach until information allows more flexibility. I believe that some protection can be obtained by requiring that a donor in this category or its DNA be proved free of any pathogen for its containment levels to be reduced. The section on "Other cold-blooded animals and lower eukaryotes" would thus have two categories. The first would be for those eukaryotes proved to be free of any pathogen which that species is known to be capable of carrying. Containment levels for this group would remain at P2 + EK1. The second category would be for all other sources and the containment levels would remain at P3 + EK2. How does the committee view this suggested classification? Further, in light of imminent availability of an EK2 host-vector system, how would the committee view raising the containment level for the first category to P2 + EK2?

With respect to the stated concerns for the use of insects, how would the committee view a stipulation in the guidelines that only insects grown for several generations under laboratory conditions be considered a source of insect DNA?

4. The last line of footnote 3 on p. 27 refers to asking CDC to prepare a complete list of Class 2 agents subdivided into low and moderate pathogenicity. In view of Dr. Sencer's March 3 reply to Dr. Stetten (copies of which were forwarded to you by Dr. Stetten's office on March 9) in which no subdivision was made, should the last sentence of this footnote be deleted?
5. There has been considerable expression of concern that prokaryotes which do not exchange genetic information with E. coli may, when they become the host of recombinant DNA, represent a particularly potent hazard for altering host characteristics as a result of any translation. The guidelines, as they appear on p. 28, do not appear to reflect this stated concern. How would the committee respond to our requiring that the minimum containment for these experiments at present be P2 + EK2 rather than P2 + EK1? This recommendation is made in view of the apparent imminence of useful EK2 systems and with the understanding that when proof is obtained that recombinant genes are not expressed and do not change the character of the host, then the committee may recommend relaxation of this containment requirement in the future.

6. Several commentators were concerned about certain definitions on p. 28 for the characterized clones of DNA recombinants derived from shotgun experiments. The concept of "free of harmful genes" was found to be troubling to many. Would the committee care to review that phrase? Further, what is the committee's response to our adding another clause to footnote 4 to make it read as follows: "The terms 'characterized' and 'free of harmful genes' are unavoidably vague. But, in this instance, before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the granting agency as guided by the advice of its Recombinant Advisory Committee." Does the committee consider this certification an appropriate and feasible function or would it suggest alternative mechanisms for this responsibility? Could the committee perform this function for any larger group of scientists other than those supported by NIH if that task befell it? Could any committee or series of them handle this problem?
7. On p. 30 the committee has listed containment levels for the use of animal viruses as the foreign source for DNA. In this section of the guidelines it is not clear whether it was the intent of the committee to permit experiments with cDNA from RNA tumor viruses. Was this the committee's intent? Would the committee object to our making explicit that only cDNA from RNA viruses which have been shown to be non-oncogenic can be used in DNA recombination experiments?
8. On pp. 30-31 of the proposed guidelines are recommendations concerning eukaryotic organelle DNA as the foreign source. There is concern about the potential contamination of purified eukaryotic organelle DNA with viral DNA. I would appreciate the committee's comment on the recommendation that embryonic primate material be permitted to be used at P3 + EK1 or P2 + EK2 and that non-embryonic primate material require P3 + EK2. I would also appreciate the committee's opinion on a requirement in this section that the mitochondria be isolated prior to DNA extraction, as a further means of reducing the hazard of viral contamination.

E. Experiments with Eukaryotic Host-Vectors

1. No issue with regard to these guidelines raised more comment than the use of SV40 DNA. Some thoughtful scientists have even gone so far as to suggest a complete ban on the use of SV40 DNA. In my view, the relatively extensive knowledge of SV40 virus provides us with added assurance that its use will yield further valuable information and, further, that sufficient sophistication exists to ensure its safe handling under the conditions developed for its use in the guidelines. I believe work with SV40 should continue under the most careful conditions. In this regard, suggestions have been made that experiments such as those described under section 2.b. on p. 37 of the guidelines should be carried out under P4 conditions instead of P3 conditions as recommended in the guidelines. What are the committee's views on this matter?
2. It is clear from the criticisms received that a number of commentators were troubled by the section on plant host-vector systems. I am also concerned that perhaps more concentrated work on this section is needed. It might be best to state in the guidelines that the setting of containment levels for this type of work will be deferred until the committee can give it more attention, supplemented by more ad hoc consultants in this field. To expedite this, I have requested Dr. Stetten to convene a subcommittee with augmented experience in plants to review this section. I would appreciate the committee's response to this suggestion.

F. Implementation

Understandably less complete than other portions of the draft guidelines, the section on implementation has aroused the largest number of comments from scientific and non-scientific quarters. These relate particularly to extension of guidelines beyond the NIH-supported community, how we can assure that research not be paralyzed by the inflexibility of excessive administrative control, how opportunity for continuous evolution of the guidelines can be protected, and the distribution of responsibilities between the investigator, his institution and community, and the NIH and other national interests. Much of the success of these

guidelines lies in the wisdom with which they are implemented. We are expanding this section and rewriting much of it as a result of review of the comments and suggestions. With one general exception, the substantive issues subject to possible change are detailed below for your consideration. Because the NIH has a special responsibility to take a leading role in assuring that safety programs are part of every program for recombinant DNA research, Dr. W. Emmett Barkley and his specially convened committee were asked to provide greater detail for safety, accident, and training plans that would be appropriate for the implementation section. That committee's recommendations are being sent to you under separate cover.

Issues for the Committee's Consideration

A. Institutional Biohazards Committee

1. There was special emphasis that the local committee include members who represent disciplines from within and without the biomedical community. What is the response of the committee to our suggesting that local committees consider including such representatives as institutional employee health officials, occupational health and safety specialists, epidemiologists, plant engineering and safety specialists, and community representatives?
2. The commentators generally approved of the requirements in the guidelines for the investigator to institute, monitor, and evaluate containment and safety practices and procedures. However, it was generally urged that this be a joint responsibility with the local biohazards committee. I believe equity of the institutions of all investigators involved in this potentially hazardous experimentation requires that there be a reasonable partnership. What is the committee's response to our recommending that the local biohazards committee share responsibility for developing appropriate safety programs and monitoring safety, accident, and training programs during the conduct of the research?

3. A number of commentators have also urged that the local committees monitor the safety, accident, and training programs at fixed intervals and that their findings be part of a record available to the public. How does the committee respond to a recommendation that the local committee monitor activities at intervals of not more than six months and that they maintain appropriate records of review activities?
4. How does the committee view our also recommending that the local committee share responsibility with the investigator to evaluate the potential hazard associated with each proposed research project and assess for each the physical and biological containment required under the guidelines? (The NIH is prepared to leave the judgment on scientific questions to its study sections as recommended in the guidelines.)

B. The NIH Recombinant DNA Molecule Program Advisory Committee

1. It has been noted that once the guidelines are issued, there may be some investigators conducting ongoing experiments that may not be in conformance with the guidelines and which must perforce cease as soon as the guidelines become effective if a switch to required containment is not possible. The minutes of your December 4-5, 1975, meeting contain the statement that "those workers who have constructed clones under the Asilomar guidelines should consult the Committee regarding their future use." When the guidelines are adopted, NIH-funded investigators in this field will be asked to respond within a given time period indicating that they will comply with the guidelines. Any investigators who constructed clones under the Asilomar guidelines, which under the new guidelines would require higher containment, will be asked to petition the NIH for a special consideration of their case. The advice of the NIH Recombinant DNA Molecule Program Advisory Committee will be sought concerning these cases. What is the committee's response to this approach?

2. The European Molecular Biology Organization Standing Advisory Committee on Recombinant DNA recently announced plans for a voluntary registry for recombinant DNA research in Europe. How would you view our recommending that the NIH maintain a voluntary registry of investigators and their institutions engaged in such research in this country?

C. Application of Guidelines beyond the NIH

Special concern has been expressed by many commentators regarding the application of the guidelines to research conducted or supported outside the NIH and its grantees or contractors. It has been urged that the guidelines be made applicable to other agencies in the Department of Health, Education, and Welfare, to the National Science Foundation, and to research conducted by the Department of Defense and other governmental departments. Other commentators urged guidelines for research in the private sector, including industry, voluntary organizations, and foundations. Others expressed concern that experiments conducted in colleges, universities, and, indeed, even in high schools, required some form of monitoring. And, finally, all agreed that in view of the potential hazards of DNA recombinant research to the biosphere, some form of international understanding on guidelines for the research was essential.

The committee, in the proposed guidelines, has suggested one means of control by recommending in the publication of research results a description of the physical and biological containment procedures practiced. We will undertake to present this recommendation to the editors of all appropriate journals. We are also prepared to take steps to disseminate news of the guidelines widely, to make them available to all who are interested, and to arrange for continued flow of information outward concerning the activities of your committee, the evolution of the guidelines, etc.

Specifically, would you please give me your opinion on whether industry, agriculture, and defense communities could and should form their own committees analogous to yours for guiding work in those sectors. (It has been envisioned that the NIH committee could function for NSF grants as well, if this is desired by that agency.)



Sample of letter to representatives of Federal agencies.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

March 30, 1976

Dear

As a follow-up to the telephone call you received from us, I am writing to invite you to attend an informal meeting to exchange information and discuss forthcoming National Institutes of Health (NIH) guidelines on research involving recombinant DNA molecules. The meeting will be held at the NIH campus in Bethesda, Maryland. It is scheduled for Thursday, April 8, from 9:30 A.M. to 12:30 P.M. in Building 1, Room 114. I have invited representatives from several other governmental agencies and departments to join us in this discussion.

Approximately two years ago, scientists engaged in recombinant DNA research voluntarily called for a moratorium on certain experiments to assess potential hazards of this research and to devise appropriate guidelines. Through their efforts, the National Institutes of Health and the National Science Foundation supported a conference sponsored by the National Academy of Sciences which was held at the Asilomar Conference Center in California in February 1975. Their actions also led the NIH to establish an advisory committee to develop guidelines for recombinant research funded by the NIH and to devise programs for assessing and controlling hazards in such research. After a year's work, the committee, in December 1975, proposed guidelines for the NIH to govern DNA recombinant research.

The proposed guidelines were reviewed at a special meeting of the Director's Advisory Committee held at the NIH on February 9-10, 1976. The meeting afforded an opportunity for the scientific community and the public to comment on the proposed guidelines. I have been reviewing these guidelines in light of the comments and suggestions made by the participants at the meeting as well as the written comments received after the meeting. After my review is completed, I will issue the final guidelines, perhaps in mid-April.

The participants at the February meeting were especially concerned that there be guidelines for DNA recombinant research not supported or conducted by the NIH. There was general agreement that the NIH should undertake to disseminate information on the guidelines as widely as possible to governmental agencies and the private sector that support recombinant research. In light of these suggestions, the meeting on April 8 will serve as an important beginning to address this public concern. I would welcome any information that you might have on ongoing DNA recombinant research. Further, at the meeting, I have arranged for a brief presentation on how the NIH intends to implement these guidelines. Your views on their applicability to your agency or other agencies and the private sector would be most helpful.

I look forward to meeting you on Thursday. I have enclosed a copy of the agenda book that was prepared for the participants at the Director's Advisory Committee meeting in February. It contains the initially proposed guidelines and supplementary material on the science of recombinant research and the potential benefits and hazards.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Enclosure

INTERAGENCY REPRESENTATIVES
NIH MEETING ON RECOMBINANT DNA GUIDELINES
APRIL 8, 1976

<u>Organization</u>	<u>Invited</u>	<u>Attended</u>
National Institute of Occupational Safety and Health	John F. Finklea, M.D. Director	John F. Finklea, M.D.
Center for Disease Control	David J. Sencer, M.D. Director	John H. Richardson, D.V.M.
Department of State	Oswald H. Ganley, Ph.D. Deputy Assistant Secretary for Advanced & Applied Technology Affairs	Oswald H. Ganley, Ph.D.
National Institute of Environmental Health Sciences	David P. Rall, M.D., Ph.D. Director	David P. Rall, M.D., Ph.D.
Office of Policy Development & Planning, Office of Assistant Secretary for Health, Department of Health, Education, and Welfare	Ms. Marian Mlay Acting Director	Ms. Marian Mlay
Office of Hazardous Materials Operations, Materials Transportation Bureau, Department of Transportation	Mr. Alan I. Roberts Director	Mr. A. W. Grella
Division of Physiology, Cellular and Molecular Biology National Science Foundation	Herman Lewis, Ph.D. Head, Cellular Biology Section	Herman Lewis, Ph.D.
Environmental Protection Agency	Roy E. Albert, M.D. Deputy Assistant Administrator for Health & Ecological Effects	Roy E. Albert, M.D.
Energy Research Development Administration	James L. Liverman, Ph.D. Assistant Administrator for Environment and Safety	Mr. C. W. Edington Mr. G. R. Shepherd
Agricultural Research Service, Department of Agriculture	Mr. T. W. Edminster Administrator	Dr. Hugo Graumann

<u>Organization</u>	<u>Invited</u>	<u>Attended</u>
Defense Research and Engineering	Col. Stanley C. White, USAFMC Assistant Director (Environmental and Life Sciences), Office of the Director	Dr. William Beisel U.S. Army Medical Institute of Infectious Diseases Dr. S. Formal Dr. L. S. Baron Walter Reed Army Institute of Research
National Aeronautics and Space Administration	David L. Winter, M.D. Director for Life Sciences	David L. Winter, M.D.
Department of Labor	Morton Corn, Ph.D. Assistant Secretary of Labor for Occupational Safety & Health Admin.	Morton Corn, Ph.D.
Division of Pathology, Bureau of Biologics, Food & Drug Administration	John C. Petricciani, M.D. Deputy Director	John C. Petricciani, M.D.
Department of Commerce	Sidney R. Galler, Ph.D. Deputy Assistant Secretary for Environmental Affairs	Sidney R. Galler, Ph.D.
Food and Drug Administration	Alexander M. Schmidt, M.D. Commissioner	Richard R. Bates, M.D. Deputy Associate Commissioner for Science

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : Director, NIH

DATE: April 9, 1976

FROM : Associate Director for
Program Planning and Evaluation

SUBJECT: The Interagency Meeting on DNA

As a result of your meeting with the agency representatives, a number of issues were raised that merit our further attention as you continue the plan for the release of the guidelines. These issues follow below:

1. Of all the agencies represented at the meeting on Thursday it would appear that OSHA, EPA, and CDC have regulatory roles that may impinge on DNA recombinant research. The possibility of litigation, for example, might arise because of certain authorities these agencies have. Therefore, you suggested that we have a review of the jurisdictions to determine if we can have a more precise definition of their potential regulatory role in this area. You also asked that there be an analysis of Peter Hutt's recommendation concerning the public health legislative authority that gives CDC power to regulate infectious materials in interstate commerce. It should be noted that several of the other agencies were more than happy to accept our guidelines, and that is a most significant contribution from the meeting on Thursday.
2. We reviewed possible NIH initiatives to alert private industry of the guidelines. It was agreed that you would call Roy Vagelos, Sid Udenfriend and Bill Hubbard. Dr. Jacobs would review other possibilities in private industry, including the drug industry. I will call Dr. Galler of Commerce to pursue with him further industry contacts. It was agreed that you should hold a meeting with private industry much as you did with the Government agency representatives. That meeting should be scheduled within the month.
3. We discussed how to proceed internally on promulgating the guidelines. Within the next few weeks you should have a final version of the guidelines. I have contacted all of the people from whom you wanted ancillary material. You should receive most of it by next week. After you have reviewed that material you can make your final decision on the last few remaining points of contention with respect to the guidelines. Next week we should also review Emmett Barkley's implementation section to complete that section of the guidelines.

With a final draft of the guidelines and a preliminary draft of your decision paper accompanying them, you could then meet with Cooper and Mathews. In your review with them the initial focus should be on the issuance of the guidelines. The question then would be "where do we go from here?" This issue might be an appropriate subject for the Health Policy Board. Your decision and the guidelines need to be reviewed to determine how you will approach the issue of regulations because that will confront you immediately after issuance of the guidelines. That needs to be carefully reviewed with Mathews and Cooper since they are responsible for regulations. In this regard you have asked Dr. Jacobs to provide a policy memorandum outlining options for you with respect to a manual issuance. That memorandum might review options of the guidelines only, the guidelines plus an I & I Memo, or the guidelines and a manual issuance.

4. The question of Government-wide adoption of the guidelines needs to be considered not only with Secretary Mathews but with Congress. As we discussed, I think it is necessary that you plan a briefing with staff on the Hill shortly before the guidelines are issued or certainly immediately thereafter. They need to be included much as we did the interagency representatives. Their views on the implementation of these guidelines Government-wide will need to be heard.
5. Further down the line we need to meet with the foundations that might support this research and we also need to have you approach the journals who publish DNA recombinant research results. Perhaps a meeting for a select group of editors could kick that effort off much as the meeting you held this past week.


Joseph G. Perpich, M.D., J.D.

Enclosure

cc: Dr. Stetten
Dr. Jacobs
Dr. Talbot



Letter of invitation to representatives of private industry
to June 2nd meeting

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

BETHESDA, MARYLAND 20014

MAY 17 1976

Dear

As a follow-up to the telephone call you received from us, I am writing to invite you to attend an informal meeting to exchange information and discuss forthcoming National Institutes of Health (NIH) guidelines on research involving recombinant DNA molecules. The meeting will be held at the NIH campus in Bethesda, Maryland. It is scheduled for Wednesday, June 2, from 9:30 A.M. till adjournment in Building 31, Conference Room 7. I have invited several other representatives from industry to join us in this discussion.

Approximately two years ago, scientists engaged in recombinant DNA research voluntarily called for a moratorium on certain experiments to assess potential hazards of this research and to devise appropriate guidelines. Through their efforts, the National Institutes of Health and the National Science Foundation supported a conference sponsored by the National Academy of Sciences which was held at the Asilomar Conference Center in California in February 1975. Their actions also led the NIH to establish an advisory committee to develop guidelines for recombinant research funded by the NIH and to devise programs for assessing and controlling hazards in such research. After a year's work, the committee in December 1975, proposed guidelines for the NIH to govern DNA recombinant research.

The proposed guidelines were reviewed at a special meeting of the Director's Advisory Committee held at the NIH on February 9-10, 1976. The meeting afforded an opportunity for the scientific community and the public to comment on the proposed guidelines. I have been reviewing these guidelines in light of the comments and suggestions made by the participants at the meeting as well as the written comments received after the meeting. A number of issues of special concern to the commentators were reviewed, at my request, by the advisory committee that developed the guidelines. I have under review the responses of the committee. After my review is completed, I plan to issue the formal guidelines with an analysis of my decision, perhaps in mid-June.

The participants at the February meeting were especially concerned that there be guidelines for DNA recombinant research not supported or conducted by the NIH. There was general agreement that the NIH should undertake to disseminate information on the guidelines as widely as possible to governmental agencies and the private sector that support recombinant research. I held a meeting with relevant agencies in the Department of Health, Education, and Welfare and with representatives from other departments of the Federal Government. A number of the representatives indicated that various departments might well adopt the guidelines where applicable.

Our meeting on June 2, similarly, will serve as an important beginning to determine the present or future interests of industrial laboratories in this type of research. I would welcome any information that you might have on ongoing DNA recombinant research. Further, at the meeting, I have arranged for a brief presentation on how the NIH intends to implement these guidelines. Your views on their applicability to your company and the private sector generally would be most helpful. The NIH is also considering the establishment of a voluntary registry of investigators and their institutions engaged in such research, and I would be most interested in your views on possible industry participation in this program.

I look forward to meeting you. I have enclosed a copy of the agenda book that was prepared for the participants at the Director's Advisory Committee meeting in February. It contains the initially proposed guidelines and supplementary material on the science of recombinant research and the potential benefits and hazards. When the final guidelines are released in mid-June, I will have sent to you copies of them and my decision.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Enclosure

REPRESENTATIVES OF PRIVATE INDUSTRY,
NIH MEETING ON RECOMBINANT DNA GUIDELINES
JUNE 2, 1976

<u>Organization</u>	<u>Invited</u>	<u>Attended</u>
Abbott Laboratories	Ira Ringler, Ph.D. Corporate Vice President Research & Experimental Therapy	Dr. Lacy Overby Director Experimental Biology
American Type Culture Collection	Dr. Richard Donovanick Director	Dr. Richard Donovanick
Bell Telephone Laboratories, Inc.	Dr. William O. Baker President	
Burroughs Wellcome	Dr. Pedro Cuatrecasas Vice President for Research	Dr. James J. Burchall Head, Department of Microbiology
Cetus Corporation	Ronald Cape, Ph.D. President	Ronald Cape, Ph.D.
Ciba-Geigy Corporation	Dr. Karl J. Brunings Vice President Pharmaceutical Division	Dr. Karl J. Brunings
Office of Environmental Affairs, Department of Commerce	W. Vern Hartwell, Ph.D. Environmental Health Specialist	W. Vern Hartwell, Ph.D.
Dow Chemical Company	Dr. D. J. Kilian Regional Director Occupational Health & Medical Research for Dow, U.S. Area	Dr. D. J. Kilian
Dupont Company	Dr. C. C. McDonald Research Supervisor Central Research and Development Dept.	Mr. C. C. McDonald
General Electric Company	Mr. Arthur M. Bueche Vice President for Research	Dr. John F. Brown, Jr. Manager, Life Sciences Branch, GE Corporate Research & Development
W. R. Grace & Company	Dr. Louis G. Nickell Vice President BioProducts Research Dept.	Dr. Louis G. Nickell

<u>Organization</u>	<u>Invited</u>	<u>Attended</u>
Industrial Research Institute	Mr. Charles F. Larson Secretary-Treasurer and Executive Director	
Eli Lilly and Company	Dr. Cornelius W. Pettinga Executive Vice President	Dr. Cornelius W. Pettinga
Manufacturing Chemists Association, Inc.	Mr. Albert C. Clark Director, Technical Section	Mr. T. Milton Freifield Asst. Technical Director, Occupational Health
Merck, Sharp and Dohme Research Laboratories, Division of Merck & Co., Inc.	P. Roy Vagelos, M.D. Senior Vice President	Dr. Jerome Birnbaum Executive Director Basic Biological Sciences
Miles Laboratories	Dr. Edward G. Bassett Manager, Department of Science Information & Communication Services	Dr. Robert Erickson Department of Science Information and Communication Services
Monsanto Chemical Company	Dr. Ernest Jaworski Agricultural Research Program	
Pfizer, Inc.	Mr. Philip Gordon Agricultural Research Program	Dr. Thomas B. Rice Mr. Philip Gordon
Pharmaceutical Manufacturers Association	Mr. C. Joseph Stettler President	John G. Adams, Ph.D. Vice President, Scientific & Professional Relations
Richardson Merrell Company	Dr. Albert Sjoerdsma Vice President for Research	
Roche Institute of Molecular Biology	Sidney Udenfriend, Ph.D. Director	Ann-Marie Skalka, Ph.D. Cell Biology
Smith, Kline and French Laboratories	Dr. Bryce Douglas Vice President Research & Development	Harry Green, Ph.D. Director of Science Liaison
Union Carbide Corporation	Dr. D. L. Heywood	
The Upjohn Company	William N. Hubbard, Jr., M.D. President	Joe Grady, Ph.D.

<u>Organization</u>	<u>Invited</u>	<u>Attended</u>
Wyeth Laboratories	Dr. Howard Tint Director Biological and Chemical Development Division	Dr. Mark Levner Biological and Chemical Development Division
Association of American Medical Colleges		Mr. Robert Carow Mr. Daniel Jones
Science and Technology Dept. British Embassy		Dr. T. M. Moynehan Attache Science
National Academy of Sciences		Dr. Elena Nightingale
Resources for the Future (Foundation)		Boyd Gibbons
		PRESS:
		Warren Leary <u>Associated Press</u>
		Myrna Zirkind, Editor <u>Blue Sheet</u>
		Rebecca Rawls <u>Chemical & Engineering</u> <u>News</u>
		Colin Norman <u>Nature</u>
		Harold Schmeck <u>New York Times</u>
		Nicholas Wade <u>Science</u>
		Al Rossiter <u>United Press International</u>
		Stuart Auerback <u>Washington Post</u>

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : Director, NIH

DATE: June 4, 1976

FROM : Associate Director for
Program Planning and Evaluation

SUBJECT: Summary of Your Meeting with Private Industry, June 2

The following points, noted here for the record, emerged from your meeting with representatives of private industry on the NIH guidelines for recombinant DNA research.

The industry representatives were pleased that their views were being considered and that they were being informed about the NIH guidelines. As Dr. John G. Adams, Vice President, Scientific and Professional Relations, Pharmaceutical Manufacturers Association, pointed out, this was the first opportunity for industry to meet and discuss this important research area.

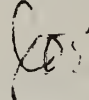
Generally, the industry representatives were receptive in reviewing the guidelines as they might apply to industrial research. They noted, however, that a number of important areas of interest to industry would have to be taken into account. In the proposed guidelines, for example, there was a prohibition of producing more than 10 liters of culture unless approved by the Recombinant Advisory Committee; and while this may be large for purposes of research at a university, it indeed could be quite small for purposes of industrial production.

Further, the guidelines are primarily concerned with pathogens, and the representative from General Electric believed attention should be given to nonpathogens that might be more commonly used in industry. Others suggested that analogs to the EK system of containment might indeed be appropriate for industrial use. And an overriding consideration for industry was the need to protect patent rights and prevent premature disclosure of research. Thus, for example, the companies might be willing to list investigators and the research labs in a voluntary registry, but might be quite reluctant to list the actual research that is ongoing.

In view of these considerations, Dr. Adams suggested that he explore with other drug industry representatives the formation of a committee to evaluate the NIH guidelines. If appropriate, the group might determine how the guidelines could be made applicable to industry in light of the special considerations raised at the meeting, and might

then relate to the NIH or other Federal agencies in applying the guidelines throughout the private sector. Again the emphasis in this regard was voluntary compliance and, if possible, no regulation by the Federal Government.

All in all, the meeting was a useful exchange that brought everyone up to date on this line of research in the private sector, on particular needs of industry that should be addressed in the guidelines, and on the desirability of a mechanism within the private sector to adopt the NIH guidelines with appropriate modifications.



Joseph G. Perpich, M.D., J.D.

Letter to Congressional staffs for purposes of briefing



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD. 20014

June 8, 1976

Don Fredrickson asked that I send you material for his meeting with you on Monday, June 14. Enclosed is the agenda book prepared for members of his advisory committee that met in February to comment on the NIH proposed guidelines. The agenda book contains a number of review articles on the subject. Further, the proposed guidelines are included, as well as a summary of them that might be better for purposes of your review.

Don Fredrickson will bring you up to date on his forthcoming decision on the guidelines when he meets with you.

Joseph G. Perpich, M.D., J.D.
Associate Director for
Program Planning and Evaluation

Enclosure

ADDRESSEES OF LETTER TO CONGRESSIONAL STAFFS, 6/8/76

House of Representatives

Mr. Henry A. Neil, Jr.
Subcommittee on Labor-HEW Appropriations

Mr. Nick Cavarocchi
Subcommittee on Labor-HEW Appropriations

Mr. Stephan Lawton
Subcommittee on Health and the Environment
Committee on Interstate and Foreign Commerce

Ms. JoAnne Glisson
Subcommittee on Health and the Environment
Committee on Interstate and Foreign Commerce

Dr. Lee S. Hyde
Committee on Interstate and Foreign Commerce

Mr. Joseph Manes
Committee on the Budget

Ms. Francie de Peyster
Office of The Honorable Tim Lee Carter

Ms. Marilyn Falik
Office of The Honorable Henry A. Waxman

Mr. Robert Maher
Office of The Honorable Paul G. Rogers

Ms. Marion Ein
Office of The Honorable James H. Scheuer

U.S. Senate

Mr. Harley M. Dirks
Subcommittee on Labor-HEW Appropriations

Mr. Terry L. Lierman
Subcommittee on Labor-HEW Appropriations

Mr. Nik B. Edes
Committee on Labor and Public Welfare

Mr. Jonathan Steinberg
Special Subcommittee on Human Resources
Committee on Labor and Public Welfare

Dr. Arthur Silverstein
Subcommittee on Health
Committee on Labor and Public Welfare

Mr. Jay Cutler
Committee on Labor and Public Welfare

Ms. Patricia Bauman
Committee on Labor and Public Welfare

Mr. Allan Fox
Subcommittee on Health
Committee on Labor and Public Welfare

Dr. Lawrence Horowitz
Subcommittee on Health
Committee on Labor and Public Welfare

Mr. Stanley Jones
Subcommittee on Health
Committee on Labor and Public Welfare

Mr. Bertram Carp
Committee on Budget

Ms. Karen J. Schubeck
Committee on the Budget

Mr. David Banta
Office of Technology Assessment

Mr. Carl Taylor
Office of Technology Assessment

Ms. Judy Robinson
Subcommittee on Employment, Poverty, and
Migratory Labor
Committee on Labor and Public Welfare

Mr. Calvin P. Johnson
Office of The Honorable Richard S. Schweiker

Others

Mr. Glenn R. Markus
Congressional Research Service
Library of Congress

Ms. Janet Kline
Congressional Research Service
Education and Public Welfare Division
Library of Congress

LETTERS RECEIVED BY
NIH REFERRING TO GUIDELINES
February 1976 - June 1976

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA 91109

DIVISION OF BIOLOGY

February 5, 1976

Dr. Donald S. Fredrickson
Office of the Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Don:

I have read with some care the recommendations of the Recombinant DNA Committee and the associated material your office has sent to me. Since you have asked me to serve as an ad hoc member on your Advisory Committee to review these recommendations, you may be interested in my reactions. Conceivably my opinions may be changed by the discussion at next week's meeting, but I feel obliged to say at this time that, in my judgement, the precautions proposed for recombinant DNA research in the committee recommendations are simply inadequate.

Although it may seem contrary, in saying this I do not wish to be critical of the committee. It is composed of outstanding people, representing a considerable spectrum of views. It is inherent then in the operation of such a committee (especially in an area so devoid of hard data) that it will seek some compromise position that will command enough support that it can perform its function. However, unfortunately, some issues in life do not brook compromise; sometimes the middle ground can be worse than either extreme.

Understandably the committee concerned itself with specific and reasonably definable potential hazards - the possible consequences of the incorporation of nucleic acids from pathogenic or tumorigenic organisms (wittingly or unwittingly) into microorganisms - and devised a graded series of precautions accordingly. It is clear that the committee did not consider that there could be any hazard from the simple incorporation of eukaryotic DNA per se into microorganisms. Thus the precautions to be taken with a clone bearing a well defined eukaryotic DNA are minimal.

I believe that this position is unjustified and indeed untenable.

First of all, I cannot believe that under these proposed guidelines the organisms can be contained. If work is going on in a hundred laboratories about the United States, performed by technicians, graduate students, etc., the organisms will inevitably escape - and will enter into the various ecological niches known to be inhabited by *E. coli*.

Dr. Donald S. Fredrickson
February 5, 1976
Page 2

The biological containment proposals are ingenious but suffer from a fatal flaw. They can all be circumvented by a simple recombination event in which the recombinant DNA is a passive participant. And regrettably organisms capable of initiating and carrying out recombination with *E. coli* abound in man (who will be doing the experiments) and his environs.

If then - as I regard as nearly inevitable - coliform (and related) organisms bearing recombinant DNA become established in various parts of our environment, what will be the consequence? I think it is unpredictable and likely highly dangerous. Prokaryotic and eukaryotic organisms (including man) coexist intimately on this planet. They interact intensely but at the phenotypic level of toxins and antibodies, etc. They do not, with any frequency (so far as I know), interact at the genetic level. Thus the prokaryotic world with its varied organisms, viruses, colicins, restriction factors, etc., and the eukaryotic world with its species, viruses, etc. have no genetic intercourse - although they use the same genetic code. One evident reason for this is the difference in control elements - promoters, initiators, terminators, etc. employed in the two domains which effectively appear to prevent expression of genetic material from one domain in the other.

Incorporation of eukaryotic DNA with its control signals into prokaryotes on an appreciable scale cannot but significantly perturb the prokaryotic-eukaryotic interaction. By recombination events these control elements could become associated with varied kinds of prokaryotic DNA. One can imagine that the viruses of prokaryotes (particularly the lysogenic species) could acquire the capacity to infect eukaryotes (consider the consequence for a eukaryotic cell of invasion by an expressible Mu-type phage - or even by a phage carrying a gene for a restriction enzyme).

As another possibility, microorganisms might acquire the capacity to serve as reservoirs for some of the common eukaryotic viruses.

One need not continue to spin out potential horror stories. The point is that we will be perturbing, in a major way, an extremely intricate ecological interaction which we understand only dimly.

A further important point, which I feel has been underestimated, is that once initiated, this course of action is most likely irreversible. We are concerned with living, self-reproducing organisms. Once set free into an appropriate environment I do not see how we could ever reverse the process (it may already be too late), should a hazard appear. The situation is thus very unlike other issues of hazard that have arisen such as the use of PCB or DDT or aerosol

Dr. Donald S. Fredrickson
February 5, 1976
Page 3

propellants. If it is discovered that these are a hazard their production can be halted and they will in time disappear. Not so with these organisms.

What course of action would I, then, recommend? I would propose that all recombinant DNA work be performed under the highest possible precaution at one site in this country (Fort Detrick?) with the most extensive possible monitoring for the escape of such organisms into the environment. (I would only allow this because of the extraordinary benefits to be gained from such research). I would at the same time launch a major program to find a much more suitable host than E. coli for such work. The qualities of such a host should be

- a) that it is found and will grow only in very selected environments
- thus if and when it escapes containment it will likely perish
and in any event can be readily monitored;
- b) that it does not exchange DNA with any organisms in the normal
human environment;
- c) that it, of course, is non-pathogenic in itself, has suitable
vectors, etc.

I do not know the ideal organism for this purpose, but I would look among the thermophiles that live in hot springs, etc.

Obviously, neither I nor anyone else can say that if the present committee guidelines are adopted, disaster will ensue. I will say, though, that, in my judgement, if the guidelines are adopted and nothing untoward happens, we will owe this success far more to good fortune than to human wisdom.

I do not envy you your task.

With best wishes,

Robert L. Sinsheimer

Robert L. Sinsheimer
Chairman

cc: Dr. Stetten

Signed in Dr. Sinsheimer's absence

College of Physicians & Surgeons of Columbia University

XXXXXXXXXXXXXXXXXX

Cell Chemistry Laboratory

XXXXXXXXXXXXXX

THE ROOSEVELT HOSPITAL
428 WEST 59TH STREET
NEW YORK, NEW YORK 10019

(212) 554-6635

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Doctor Fredrickson:

Your letter of January 29, signed by Dr. Charles R. McCarthy, concerning the meeting of the Advisory Committee on February 9-10, reached me only today; too late, unfortunately, for me to arrange to be in Washington at that time. In any event, it is not quite clear to me why this letter was sent to me, unless I am meant to make a brief statement as a "member of the public." I am both more and less than that: less, because I represent no lobby or common interest group, nor have I been elected to represent any segment of the public; more, since I am one of the early workers on DNA, a member of the National Academy, and a recent recipient of the National Medal of Science.

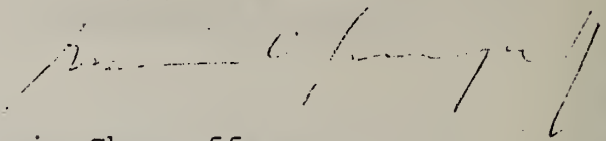
I have alluded only once in print to the dismal problem of DNA recombination experiments, and I take the liberty of enclosing a reprint of a recent article of mine in which I have marked the passage in question.

Beyond that I can only express my strong dismay about the whole problem, as it has been formulated, restricted, expanded, and drowned in a verbiage of silly claims and counterclaims. If I were asked what solution I suggest, it would be that there should be a complete stop on all forms of experimentation with artificially produced DNA recombinants that could in any way represent a potential danger to animal or plant life. Such a prohibition would have to have the sanction of the international bodies representing the biological and exact sciences, such as IUB or IUPAC. As far as this country goes, such a prohibition -- perhaps limited to a period of, say, two years, to permit a cooling off, accompanied by discussion and reflection -- would presumably require congressional action.

Received 2/9/76

I should be grateful if I could be kept informed of further developments, perhaps not so much in my capacity as a member of the public, but as a scientist concerned with the opinions about science held by the public.

Sincerely yours,



Erwin Chargaff
Professor Emeritus
of Biochemistry

EC:eh
Encl.

Saint Mary-of-the-Woods College
Saint Mary-of-the-Woods, IN 47876
February 10, 1976

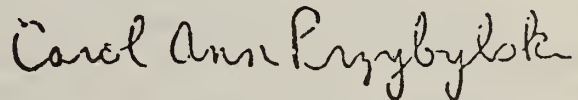
Dr. Donald Frederickson, Director
National Institutes of Health
Bethesda, MD 20014

Re: January 30, 1976 letter to members of FASST concerning proposed guidelines for research on recombinant DNA

Dear Dr. Frederickson:

As a FASST member interested in biomedical technology I wish to share my views on the above-mentioned proposed guidelines. I feel it is wise to classify the DNA experiments according to increasing potential hazard and also agree that procedures should be appropriately strict. Progress is great but only as it minimizes danger to the environment. We hope, I assume, not to create dangerous imbalances in nature, and will not, if the suggestions before the Advisory Committee are favorably considered. Perhaps, in a year or two, then, the experiments now deemed so hazardous as to be shunned may be tried on a limited basis in a tightly-controlled environment.

Sincerely yours,



(Miss) Carol Ann Przybylski

CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF EMBRYOLOGY
115 WEST UNIVERSITY PARKWAY
BALTIMORE, MARYLAND 21210
TELEPHONE: 467-1414

February 12, 1976

Dr. Donald Frederickson, Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Frederickson:

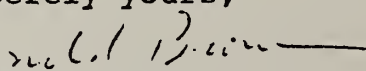
I wish to summarize briefly why I believe that the "Proposed Guidelines for Research Involving Recombinant DNA Molecules" are too strict and too inflexible. I refer specifically to the part which requires at least EK2 biological containment for recombining plant, vertebrate and viral DNAs. Since there are no EK2 vectors, nor a clear mechanism of how EK2 will be certified when they exist, this requirement effectively places an indefinite moratorium on gene isolation research with these organisms. There are two ways that flexibility could be introduced without compromising safety. First, instead of categorically requiring EK2 for these experiments, give investigators a chance to present their experiments in detail to some objective committee. There are certain kinds of genes and certain ways of doing shotgun experiments which even the strictest critic might find totally acceptable to be carried out under EK1. If the committee is not fully satisfied with the safety precautions of the scientist, then they will require him to wait until EK2 systems are available.

Second, make some government P4 facilities available with trained technicians for scientists who wish to do shotgun experiments. Require that the clone containing the gene be identified and characterized at the P4 facility. At that point the guidelines permit the investigator to work with it in his own laboratory. This is a safe way to use existing (EK1) systems.

Finally, I urge you to devise methods for the review and revision of the rules as often as possible.

I thank you again for the opportunity to state my opinions before you and the Advisory Committee.

Sincerely yours,


Donald D. Brown, M.D., Staff Member
Carnegie Institution of Washington

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA 91109

DIVISION OF BIOLOGY

February 12, 1976

Dr. Donald S. Fredrickson
Office of the Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Don:

I have been reviewing my letter to you of February 5 in the light of our recent meeting to see what statements I might wish to change or what I might add.

I still am highly inclined to caution. The various potential hazards cited, for instance, by Wallace Rowe - while troubling enough - took into account only the potential for mischief that might be produced by extant gene combinations, when transported into or expressed within unfamiliar habitats. Little attention seems as yet to have been given to the potential hazards that might derive from the wholly new combinations of genes we shall create (or afford new opportunity for creation) by recombinatory events.

I am not persuaded by the argument that the opportunities for the creation of these potentially dangerous gene combinations already exist in Nature - at some unknown frequency. Such events may indeed occur sporadically - and with what consequence? (New viruses do appear from time to time - whence?). Clearly we will be increasing the opportunities for such gene combinations by many, many orders of magnitude. I believe my analogy with the peril from radiation (despite our adaptation to background radiation) is apt.

Nor am I persuaded that "biological containment" is adequate. I do not rest easy on Roy Curtiss' 10^{-19} style numbers because I expect other events with which he has not reckoned will come into play at much higher probabilities (i.e. the original cloning culture will be contaminated; cells will lyse in a pharynx and the DNA be taken up by another organism, etc. etc.).

The irreversibility of the process - which can only increase over time as more and more laboratories and persons become involved - continues to disturb me.

Let me try to make a few suggestions as to directions I would think might allow research to proceed yet provide a broader safety belt. I think it easiest to work backward from the conceivable applications (benefits).

Dr. Donald S. Fredrickson
February 12, 1976
Page 2

One application would be the synthesis of desired proteins (e.g. proinsulin) in microorganisms. Economically, I accept that this must be done in a living cell (i.e. chemosynthesis, ribosomal synthesis, etc. seem too complex and expensive). A gene for proinsulin with proper prokaryotic control signals would be synthesized chemically (or conceivably made as a cDNA from an mRNA once isolated). The defined piece of DNA could then be inserted into a plasmic or virus - but not destined for *E. coli*. I would propose the use of some micro-organism which has no association with man (or other animals), which has a very restricted range of genetic exchange and, ideally, a very limited biological niche of viability.

I would also propose the development of procedures that could be maximally automated so as to minimize all possible contact of such organisms with our biosphere.

Another current application of great interest is the isolation (via shotgun techniques) of specific pieces of eukaryotic DNA. Here my objection is to the irreversible incorporation of any such undefined DNA into free-living ubiquitous bacteria. I would suggest that it would be safer - and far more potentially reversible - to incorporate such DNA into an animal virus against which we already have a viable defense - for example, cowpox. All persons and animals who might come in contact with such organisms could be vaccinated. If some hazard were perceived, all infected animals or tissue culture cells could be exterminated (we have coped with outbreaks of animal disease before). The genetic gap between prokaryotes and eukaryotes would not be bridged.

(I presume an analogous approach could be taken for experiments within the plant kingdom - obviously without vaccinations).

My thought then is to approach each desired objective in as specific (and safe) a manner as we can think of rather than to attempt a global approach which inevitably seems to leave uncovered opportunities for experiments of large (in my view) potential danger.

I regret that the format of the meeting did not really permit an exploration of this point of view with the committee as I would be interested in their reactions (I did have an opportunity to discuss some of this privately with Paul Berg).

I appreciate the difficulties you are in with respect to any modification of the guidelines from the Recombinant DNA Committee which has worked long and hard for a year; also with respect to the whole biological community, many members of which simply do not believe a hazard exists. Almost any stricter proposal than

Dr. Donald S. Fredrickson
February 12, 1976
Page 3

the proposed guideline will surely have to be accompanied by a very strong statement of concern about hazard and responsibility to safeguard the public health.

One other aspect of the discussion at the meeting disturbed me - and I must admit that my suggestion - for very selective, ad hoc guidelines and permissions - plays into it. I could sense an inclination among the more legally minded toward a path which would ultimately lead to a required approval prior to an experiment. This troubles me greatly for it would be a dire impediment to science. Yet perhaps this will be a necessary accompaniment to the synthetic mode of biological research, in the absence of feasible containment. We have only one habitable planet.

With best wishes,

Bob

Robert L. Sinsheimer
Chairman

cc: Dr. DeWitt Stetten, Jr.

BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77025

DEPARTMENT OF VIROLOGY AND EPIDEMIOLOGY
(713) 790-4444

February 12, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Md. 20014

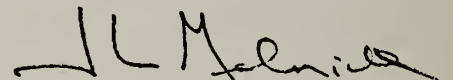
Dear Dr. Fredrickson:

I am enclosing a typewritten copy of the remarks that I made at the meeting of the Advisory Committee to the Director on February 10.

I have attempted to formulate a procedural mechanism that keeps the group of DNA experts together but on rotational basis. To avoid the chance of their being a self-serving group, a policy making Advisory Council is placed above them. Procedures are incorporated for flexibility so that the regulations can be modified as required by the acquisition of new knowledge. A mechanism is incorporated so that concerned scientists and others can have a forum before the Committee on a regular schedule.

Since returning home, I have heard that chick cell genes are growing well in E. coli. Before matters get out of hand by investigators not privy to the NIH discussions, I believe that your office should move rapidly (but deliberately) in coming to a decision on how work in this critical area should move forward safely.

Kind regards,



Joseph L. Melnick

JLM:jmb
Enc.

Because of the important but uncharted areas opened up by recent developments in Recombinant DNA research, the following procedures are recommended, to be administered by the Office of the Director of NIH.

The Director shall appoint a Technical Committee on Recombinant DNA, to be made up of 15 experts in the field. At the beginning, five will be appointed for one year, 5 for 2 years, and 5 for 3 years. At the end of each year 5 new members will be appointed for a 3-year term. The committee will meet every 4 months. Its functions will be:

- (1) To review the current regulations (euphemistically heretofore called guidelines) and to suggest modifications as new information becomes available.

- (2) To plan model experiments to be carried out in the appropriate NIH containment facilities. These experiments will be planned to yield data to determine the next steps that can safely be taken to advance our knowledge of Recombinant DNA. The data and their analysis shall be published as each forward step is taken.

- (3) To review Recombinant DNA research grant and contract applications submitted to NIH and to evaluate them for scientific merit and for adherence to safety standards.

The Technical Committee will report to the Recombinant DNA Advisory Council, which serves as the policy-making body. It receives the reports and after deliberation recommends action to the Director of NIH.

The Director appoints the members of the council, to be made up of 8 scientists not working directly on DNA Recombinants plus 4 representatives of the public at large. The 8 scientists will be drawn from leaders in related fields of biomedical research (such as genetics, bacteriology, virology, botany, infectious diseases, public health, etc.) and the 4 non-scientists from leaders in areas of public concern (such as bioethics, law, sociology, medical history, etc.).

Each institution receiving NIH funds will be required to appoint a local committee on biohazards, which will be expected to become familiar with the Recombinant DNA Safety Regulations promulgated by the NIH Technical Committee and approved by the Advisory Council. No research grant or contract will be considered by NIH in the area of Recombinant DNA unless the local committee certifies, to the satisfaction of the Technical Committee or its designee, that the principal investigator and the local institution meet these Safety Regulations. If the grant is awarded, the local committee will periodically inspect the laboratory of the principal investigator and submit an annual report of these inspections to NIH to assure that the Regulations are being carried out.

Except when the Technical Committee is considering Applications for Research Grants, its meetings shall be open to the public. Time will be provided to hear the opinions of scientists and non-scientists who feel that the regulations should be modified. These discussions will be summarized and evaluated by the Technical Committee for consideration by the Advisory Council.

CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF EMBRYOLOGY
115 WEST UNIVERSITY PARKWAY
BALTIMORE, MARYLAND 21210
TELEPHONE: 467-1414

February 12, 1976

Dr. Donald Frederickson, Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Frederickson:

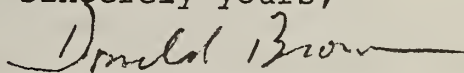
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Second, make some government P4 facilities available with trained technicians for scientists who wish to do shotgun experiments. Require that the clone containing the gene be identified and characterized at the P4 facility. At that point the guidelines permit the investigator to work with it in his own laboratory. This is a safe way to use existing (EK1) systems.

Finally, I urge you to devise methods for the review and revision of the rules as often as possible.

I thank you again for the opportunity to state my opinions before you and the Advisory Committee.

Sincerely yours,



Donald D. Brown, M.D., Staff Member
Carnegie Institution of Washington

DDB:ar
cc: Dr. D. Stetten

FRIED, FRANK, HARRIS, SHRIVER & KAMPELMAN

FELIX S. COHEN (1932-1953)

RICHARD B. BERRYMAN
DAVID E. B'RENBAUM
JAMES B. BLINKOFF
PETER D. EHRENHAF
MILTON EISENBERG
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PATRICIA ROBERTS HARRIS
WILLIAM JOSEPHSON
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HENRY A. HUBSCHMAN
JAY R. KRAEMER
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SAMUEL A. SIMON
WILLIAM E. SUDOW
JILL WINE VOLNER
HOWARD M. WEINMAN
W. RICHARD WEST, JR.
KAREN HASTIE WILLIAMS

FREDERICK SASS, JR.
COUNSEL

February 13, 1976

OUR REFERENCE

Donald S. Frederickson, M.D.
Director
National Institutes of Health
Bethesda, Maryland 20014

Re: Recombinant DNA Guidelines

Dear Don:

Let me make somewhat more precise my suggestion at your Advisory Committee meeting on February 9 to include in the NIH Recombinant DNA Guidelines provisions for compensation. I believe such provisions might appropriately be added to Part IV B which sets forth the responsibilities of each grantee institution to certify to the quality of physical containment facilities. See pp. 43-45, Jan. 1976 Proposal.

I propose that you expand the institutional responsibility to include a certification that a workmen's compensation or similar insurance scheme is in force at the grantee institution and covers persons injured in the course or as a result of such experiments. Such compensation should be awarded without the necessity for proof of fault or negligence.

You may also wish to assure yourself that such compensation is available at the National Institutes of Health and other Federal installations conducting recombinant DNA research.

Donald S. Frederickson, M.D.
Page Two
February 13, 1976

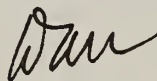
In this connection, let me direct your attention to paragraph 3 of the Statement of Commissioners Karen A. Lebacqz and Albert R. Johnsen to the Report and Recommendations of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research printed in 40 Fed. Reg. 33530 ff. (Aug. 8, 1975). The Statement appears at pages 33550-51 and a copy of those two pages is enclosed herewith.

I recognize that it may be premature to impose such a requirement in the initial Guidelines. I would nonetheless urge that, at a minimum, grantee institutions be directed to develop such a compensation program and, further, that the matter of compensation be placed on the agenda of whatever group may have continuing responsibility for advice with respect to modification and amendment of the Guidelines.

As a technical matter, I would suggest with regard to Part IV B that the certifications both as to compensation and as to containment facilities be made by and in the name of the institution itself and not by a biohazard committee.

Warm personal regards.

Sincerely,

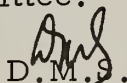


Daniel M. Singer

DMS/kk

enc.

P.S. I would very much appreciate having a copy of the text of your opening remarks delivered February 9 following your introduction of the members of your Advisory Committee.



D.M.S.

Yale University *Box 1937 Yale Station, New Haven, Connecticut 06520*

DEPARTMENT OF MOLECULAR BIOPHYSICS
AND BIOCHEMISTRY

(203) 436-3611

February 13, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

I have just completed reviewing the Proposed Guidelines for Research Involving Recombinant DNA Molecules. As co-author of the original letter (*Science* 181, 1114 (1973)) which started to focus attention on the possible hazards of recombinant DNA molecules, and which led to the formation of the Berg committee, I feel that the proposed guidelines are well considered and provide adequate precautions for future research. I urge you to adopt them.

Yours sincerely,



Dieter Söll
Associate Professor
Molecular Biophysics

DS:ml
Encl.

DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS
HARVARD MEDICAL SCHOOL
25 SHATTUCK STREET
BOSTON, MASSACHUSETTS 02115

13 February 1976

Donald S. Fredrickson, M.D.
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

My immediate inclination after the hearings at NIH was to send , once again, our written comments on the issues in question. Thinking over this course of action made me hesitate and then decide to scrap such an approach. You are aware, I hope, of our position. Likewise for the views presented by Drs. Berg, Hogness, Singer, and Curtis. At both the industrial and university level molecular biologists await your decision.

The brief respite since the hearings has given me a rare moment to stand back and look for an overview of events occuring since Woods Hole and the issues in question. I was struck by many things, two of the most prominent being:

- 1) that America is once again, as in the case of atomic power, opting only for development rather than development, leadership, and control of a new technology; and,
- 2) that through out this controversy, the various sides have never sat down together and delt with the issues point by point. Such does not happen via the press or during public hearings.

My own future involvement the with the recombinant DNA molecule issue is questionable since past efforts have caused me to neglect both teaching and research responsibilities. It seems that there is no academically acceptable avenue for involvement in the development and style of future studies with recombinant DNA molecules. This somehow seems wrong since we all agree that it will have a heavy impact on both science and society. I would be interested in hearing any thoughts you might have on this matter.

Please feel free to contact me if I can be of any future help.

Sincerely,



Richard Goldstein
Assistant Professor

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : Dr. Donald Frederickson,
Director, NIH

DATE: February 13, 1976

FROM : Maxine Singer
Head, Nucleic Acid Enzymology Section
Laboratory of Biochemistry, DCBD, NCI

SUBJECT: Proposed Guidelines For Research Involving
Recombinant DNA Molecules

During the meeting on February 9 and 10 I did not consider it appropriate for me to make comments on the substance of the proposed guidelines. It was my understanding that my primary responsibility to the proceedings was as an information source and indeed, I believe that to have been the most important contribution I could make.

Nevertheless, you will not be surprised to learn that I do have some specific views concerning the decisions you must now make.

A. The Experimental Guidelines

The LaJolla version represents a cautious approach to the experiments. Overall, I believe the recommendations afford a high level of confidence that those experiments which appear to present very serious potential hazards will be satisfactorily contained, either because they are not to be carried out at all, at present, or because of the requirement for P4 and/or EK3. Furthermore, given the evidence that expression of eukaryote genes in E. coli hosts is either unlikely or at best inefficient under current experimental conditions, it seems that nature itself is ensuring a "go slow" approach.

In my view, the proposed guidelines do not seriously compromise the ability to undertake the bulk of the scientifically important problems.

There is one substantive matter in the guidelines about which I have some reservations. A reasonable argument can be made for restricting use of SV40 as a vector solely to P4 conditions, when viral particles are produced. Indeed, a reasonable argument can be made for avoiding the use of SV40 as a vector altogether, when viral particles are produced. Thus, while I find the present recommendation acceptable (primarily because ignorance does not permit positive decisions, even in the face of reasonable arguments) I would also find a more conservative guideline on this point acceptable.

B. Implementation

This is an area in which I have serious questions about the adequacy of the proposed guidelines, as well as some of the alternatives discussed during the meeting. Perhaps it has been a naive dream, but throughout the last several years I have hoped that the required implementation and enforcement could be achieved with a minimum of centralized and overbearing bureaucracy. The atmosphere engendered by such a situation is not conducive to open, high quality scholarship. Furthermore, the evidence from unrelated areas does not afford much confidence in the efficacy of centralized, highly structured regulation. Nevertheless, the proposals contained in the guidelines seem to me insufficient in several ways.

1. The structure which relies on the primary responsibility of the investigator, with existing study sections as review bodies, is basically sound and administratively efficient. It could, however, be more effective if the proposed role of institutional or regional Biohazard Committees was strengthened. The guidelines take a negative approach to the local Committees (p. 44) in stating that it is not their responsibility to determine required containment. Perhaps the guidelines should state that local committees may, with the investigator, undertake determination of required containment. This would still allow flexibility, and would permit the Committees to function in a variety of ways in response to particular local situations. In fact, I think it likely that universities and other institutions will insist on some official interest in these determinations since they will clearly bear responsibilities in case of possible injury. Given this inevitable interest of the universities and institutions, it seems to me that their role, combined with that of the investigators, the study sections, and local committees affords a series of independent checks on compliance with the guidelines.

2. The important role of peer pressure in compliance will be most effective if the recommendations concerning publication are heeded. Perhaps a special effort at soliciting the cooperation of the Journals could be made, by the NIH.

3. I believe it essential that the NIH assume leadership in assuring that the guidelines apply to all possible situations, not just NIH grants. And this should be done simultaneously with your decision on the guidelines. First, it must be clear that NIH contracts also will be covered.

Second, while I understand that it is not within the official NIH mission, informal efforts might obtain the public concurrence of various other relevant organizations, ideally at the time you announce your decision. For example:

- a) Other federal agencies which support biological research.
- b) Private funding agencies, such as the American Cancer Society.
- c) Major organizations of the teachers of biology in secondary schools.
- d) Relevant scholarly societies such as the American Society of Microbiologists, the Biophysics Society, the American Society of Biological Chemists, the Genetics Society.
- e) Either the relevant trade association, or major pharmaceutical companies.

On the basis of the goodwill we have all experienced in this matter over the past few years, I would hope for cooperative responses from such groups, except perhaps for the scholarly societies which tend to be reluctant concerning public policy matters.

C. Finally, I would like to share with you my own sense of urgency concerning your decision. My colleagues have, by and large, been patient through these several years of thought and debate, even when they have not completely shared the concerns that motivated some of us. Seemingly endless levels of debate have now discouraged many of them. If we want their cooperation in this, and similar problems to come, we must promptly have a definitive resolution.

The "go-slow" argument expressed by several attending your meeting is, I believe, what we have had and would have, with the proposed guidelines. The discussions of the last few years resulted in a "go-slow" situation: the guidelines, insofar as they require P4 or EK3 for the most worrisome permissible experiments, will result in the continuation of a "go-slow" policy.

The meeting itself, both in conception and organization was a logical, useful, and important addition to the "process". The manner of your chairmanship was to my mind, admirable in every way. I would especially like to express my gratitude for your continuation of premises that have been the basis of my own activities in this area,

Dr. Donald Frederickson

Page 4

namely, that matters of this sort can be resolved in a rational and scientific manner, and that our community, while preserving its interests and freedoms, can be both responsible and trustworthy.

Sincerely yours,

Maxine Singer

Maxine Singer
Nucleic Acid Enzymology Section
Laboratory of Biochemistry
National Cancer Institute

THE UNIVERSITY OF TEXAS
HEALTH SCIENCE CENTER AT DALLAS

CHARLES C. SPRAGUE, M.D.
PRESIDENT

SOUTHWESTERN MEDICAL SCHOOL
GRADUATE SCHOOL OF BIOMEDICAL SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES

February 16, 1976

Donald S. Fredrickson, M.D.
Director
Public Health Service
National Institutes of Health
Department of Health, Education, and Welfare
Bethesda, Maryland 20014

Dear Don:

As you requested, I shall put down in writing some of my thoughts with respect to the Directors Advisory Committee meeting regarding the recombinant DNA research guidelines.

May I say at the outset that you and your colleagues at NIH plus a small group of investigators are to be commended for the manner in which this entire problem has been handled. I think you have established, in the eyes of the public, the image of a governmental agency that is as interested in obtaining and considering the best in public opinion as it is in having scientific input.

In developing the guidelines to their present state, the representatives from the scientific community, as well as the representatives from the NIH, have demonstrated not only thoroughness, thoughtfulness and reasonableness with respect to the scientific aspects of the guidelines, but they have shown a remarkable degree of concern for the public good. While some criticism was directed at the composition of the NIH Advisory Committee, with it being heavily weighted in favor of scientists with vested interests, I daresay that a more broadly representative committee probably would not have come up with guidelines significantly more "conservative" unless, indeed, they would have wished to make them so restrictive that they would have, in fact, precluded further research in the field. Such action would, in my opinion, have been both unfortunate and unwarranted.

While I feel incapable of commenting on the exact wording or even the scientific merit of some of the judgments made by the advisory group with respect to the guidelines, I do have some general comments to make. There seems to be an obvious

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Donald S. Fredrickson, M.D.
February 16, 1976
Page 2

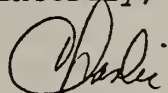
need to make more explicit some statements in the guidelines that were implicit in the current version. There may even be the desire or need to make some substantive changes, or additions, in terms of review procedures to be followed both locally and nationally in the implementation of the guidelines. Although I stated at the meeting that I felt that you should err on the conservative side in the final version, I would hasten to add that I believe the persons responsible for developing the present guidelines had that in mind all along and when there was any reasonable doubt as to the right decision they always seemed to be overly conservative perhaps and go to more restrictive language.

As important as it is to build in adequate safeguards at the outset, it is equally important to provide a mechanism to revise the guidelines, at frequent intervals if necessary, to take into account new information and knowledge. In that regard I have two specific suggestions: (1) rather than have the advisory committee meet "at least annually" as stated in the present guidelines, I would suggest meeting 3-4 times a year. (2) To define as precisely as possible many of the risks about which there is such speculation and controversy at present, I think the NIH, by contract research and/or in-house research, should see that certain critical experiments, some of which were described by Dr. Rowen, be carried out as soon as possible.

Although granted it is very much a "gut reaction" I feel fairly comfortable that if, indeed, investigators throughout the world would adhere to the guidelines as they are finally promulgated, there would be minimal chance of something going awry. One of my major concerns, probably the major concern, is how do we get everyone to play by the same ground rules. Frankly, I am not as concerned about our highly competitive pharmaceutical firms as I am with irresponsible investigators in certain countries where there is little or no concern within the government or the public regarding the hazards of such research. I do hope that your advisory group will give this problem area its most serious attention.

Lastly, I would like to thank you and congratulate you on the exemplary manner in which you handled a most difficult assignment. May I add that those of us "in the field" are much gratified at the sound and vigorous leadership you are providing the NIH.

Sincerely,



Charles C. Sprague, M.D.
President

[459]

ccs:db

STANFORD UNIVERSITY MEDICAL CENTER

STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY
STANFORD UNIVERSITY SCHOOL OF MEDICINE

Area Code 415
497-6161

February 17, 1976

Dr. Donald Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Don,

Perhaps it would be useful to you if I commented on some of the suggestions that were voiced at the recent meeting of your Advisory Committee (Feb. 9-10, 1976) that considered the guidelines for research on recombinant DNA molecules.

I. It would, in my opinion, be most unwise to transform the guidelines into a legalized set of regulations, as suggested by Peter Hutt. I doubt if he appreciates either how fast the data base for the guidelines can change from its present impoverished state, or how approximate is their present formulation. The guidelines are, by their very nature, bound to contain discrepancies. This is true because: (1) they attempt to cover the entire range of possible experiments with recombinant DNA molecules and hence include all life forms on this planet; and (2) they attempt this coverage with a relatively small number of biological boundary lines for classifying this vast area of experimentation. Under these conditions, it is inevitable that particular experiments in which DNA from a given organism is inserted into a given vector will be misplaced in the guidelines. Moreover, both the physical and biological containment components of the classification will undergo rapid change as new techniques and host-vector systems are developed.

We therefore need flexible administrative mechanisms whereby the guidelines can be easily modified and that allow rapid consideration of individual experiments that do not fit the classification system. Casting the guidelines in the legal format of regulations can only inhibit that flexibility and thereby retard both the experimentation and the adoption of rational safeguards.

II. In advising existing or potential principal investigators about the guidelines for research with recombinant DNA molecules, the NIH should explicitly state: (1) that the guidelines are not hard and fast rules, but are instead guides to aid the principal investigator in estimating the risk associated with a given experiment and in designing appropriate safeguards for that experiment; and (2) that the NIH urges the principal investigator to design alternate safeguards, and to present information that in his or her opinion alters the estimate of risk indicated in the guidelines. While reserving the right not to fund particular experiments that it deems too hazardous, the NIH should not get boxed into the position where it must defend a particular "cookbook of recipes" defining

how basic research in this area is to be carried out.

As one who has been through each phase in the formulation of the proposed guidelines, I cannot emphasize too strongly the tendency to dictate how experiments must be carried out in order to make the document specific, i.e., "to plug loopholes". Because this tendency violates a basic principle of good research, particularly as the hazards that induce it are speculative, it can be respected and therefore be useful only if the document is considered as a guide. If on the other hand it is considered as a set of rules by the NIH, it will be treated with disrespect, and, in my opinion, its inherent discrepancies will become a target for legitimate ridicule and confrontation.

The containment principles in the guidelines will be effective only if they earn the respect of the scientists carrying out the experiments and this respect cannot be gained by fixed rules that in many instances appear arbitrary. By contrast, it can be gained if NIH makes clear that it wishes to involve these scientists in the decision making process, acknowledges possible discrepancies, and will conscientiously consider alternate evaluations and procedures. Hence, Peter Hutt's contention that it is fictional to consider the document as a set of guidelines rather than rules is an inversion; rather I think it is a figment of legalistic imagination to believe that effective safeguards can be attained by the imposition of a set of fixed rules applied to such a vast area of basic research, and in the absence of demonstrated hazards.

III. Finally, I believe it is vital that the proposed guidelines be accepted by the NIH without further delay. The previous delays have engendered a confusion that has been harmful in many ways. Scientists wishing to carry out certain experiments have not been able to plan the appropriate safeguards, or feel they have been misled in regard to the plans they have made. And I should imagine that the concerns of the public and Congress are exacerbated unnecessarily by continued delay. Given that they are acknowledged as approximations and are considered as guides, not rules, I think the guidelines should be promulgated in their present form immediately. I do not think that revision of the containment conditions recommended for the different classes of experiments should be considered at the next meeting of the NIH Recombinant DNA Molecule Program Advisory Committee in April, much as I and others on the committee would like to advocate certain changes in the near future. Rather, I think the wisest course is to proceed with the present guidelines along the lines given in I and II, above, and allow some time for the development of the flexible procedures we require, and for everyone, including the study panels, to become acquainted with the guidelines.

I hope these comments are of some use to you. I have enclosed a copy of the talk I gave to your Advisory Committee on Feb. 9, 1976, for the record.

Cordially,

A handwritten signature in dark ink, appearing to read "D. Hogness", with a stylized flourish at the end.

David S. Hogness

February 17, 1976

Dr. Donald Fredrickson, Director
National Institutes of Health
Bethesda, Md.

Dear Dr. Fredrickson:

The following comprises my comments and suggestions concerning the proposed Guidelines for Research Involving Recombinant DNA Molecules.

The decisions you must make in regulating recombinant DNA molecule research are of critical importance, not only because they will provide a model for other countries in regulating research within their borders, but because they are the fulfillment of a process which will be viewed as a case study in the genesis of science policy for many years to come.

In the guise of the recombinant DNA molecule controversy, scientists have been provided with a critical opportunity to retain control over their own fate, as the outcome may well determine the degree of outside intervention scientists will have to deal with in the future. The extent to which they show themselves disinterested and responsible will provide the rationale for whatever degree of control they maintain. The question has become, not whether research will be regulated, but who will control it.

For these reasons, it should be emphasized that the construction of these regulations is a public act.

Whatever the outcome, in terms of specific guidelines, it is almost certain that they will turn out to have been inadequate or excessive in some respects, as data accumulates. Thus it is vital that the guidelines be readily modifiable for the first few years, at least.

In light of the above, it must be pointed out that there is an interaction between conservatism and flexibility - if modification is a realistic expectation, then strict guidelines may be recommended with confidence that they may be altered if it subsequently appears that they are overzealous. I therefore strongly support the principle, expressed in the guidelines and in the Asilomar statement, that "Containment should be high at the

start and modified subsequently only if there is a substantiated change in the assessed risks".

In order for any useful evaluation of the adequacy of the guidelines to occur, there must be more information about both the degree of hazard and the adequacy of containment. For this reason I urge that NIH should pursue, under stringent safety precautions, experimentation bearing on these questions. Of particular interest is the question of whether eukaryotic genes are ever expressed in prokaryotes. If not, then the risks of experiments inserting eukaryotic DNA into prokaryotes may be judged to be considerably lower than would be the case if such genes were indeed expressed.

Physical Containment - No form of physical containment can be considered totally effective - we must assume that a breach will occur eventually. I am not convinced that P1 and P2 as generally practiced will provide substantial protection. Any experiment in which escape of laboratory organisms is judged at all hazardous should not depend on these means.

Nonetheless, technique is the key to safe procedure, but technique is highly dependent on the attitude of the worker. Dr. Shaw's suggestions regarding consent procedures could possibly serve a dual role in this regard. Workers could be required to sign a consent form indicating that they understand the risks of the research and are trained in strict microbiological technique. Such a procedure would provide a mechanism for informing workers of the nature of the risks to which they and the public will be exposed, as well as for certifying their competence. Further, it might serve as a "consciousness-raising" device, periodically reminding workers that they are working with hazardous materials. This procedure might help to avoid the sloppiness and casual attitude that often arises from routine handling of dangerous substances.

Biological Containment - In many respects biological containment is the key to our hope of avoiding release of dangerous organisms, as no form of physical containment is entirely effective. I share the misgivings of many of the public witnesses about the use of any form of E. coli as a standard experimental organism. I urge that NIH make a concrete commitment to the development of a vector-host system with a less ubiquitous natural distribution, and more limited capacity for exchange of genetic information with other organisms.

Further, serious thought should be given to methods for inducing researchers to switch from use of E. coli, as it is clear that those accustomed to work with E. coli will be reluctant to change.

I do not propose an immediate ban on E. coli, because, although I am not convinced that Dr. Berg's claim that it will take an-

other 25 years to develop another organism to the level of understanding now attained with E. coli is correct, I recognize that it will take time, and it is certainly not in the public interest to drive recombinant DNA research underground with such a ban.

Perhaps a time limit such as that suggested by the Boston Group is advisable, although it would certainly have to be negotiable--target dates should certainly be possible. It is important to avoid slipping into a habit of thinking only in terms of E. coli, and becoming complacent about alternatives. Unfortunately, the fact that the guidelines are written entirely in terms of E. coli encourages assumptions of this nature.

The use of "safe" vector-host systems (EK2 and 3) does not entail the quantum leap in difficulty and expense of experiments that results from the increase of physical containment. The inconvenience resulting from restricting experimentation to these organisms is simply that genetic diversity of these systems is limited, and some experiments could not be performed within them. I suggest that all experimentation should be routinely restricted to the safest available host-vector systems - EK2 or 3 level as soon as available (EK3 systems are simply the same EK2 systems subjected to further testing, and thus are no different to work with). The use of any but the safest available system should be permitted only by a petition to the Program Advisory Committee delineating why the experiments cannot be modified to permit them to be performed in safer systems, and why they should not be postponed until a suitable EK3 or 2 level system can be developed. Such a procedure should provide a strong incentive for remaining within the safest possible systems, and for the development of a diverse array of safe host-vector systems.

I also would strongly discourage the use of any virus known to infect humans, such as SV40, as the effects of such infections may not be known for a generation. I would urge a procedure such as that suggested above, in which experiments with SV40 or any virus infecting humans would be allowed only if it were demonstrated that there was no other way to obtain the information and that experiments should not be postponed, and then permitted only under the most stringent conditions.

Implementation - The method of implementation of these guidelines is an important consideration in judging how effectively the scientific community has dealt with this crisis, for if they are loosely or irregularly applied, the past two and a half years of soul-searching and altruistic behavior will have been in vain.

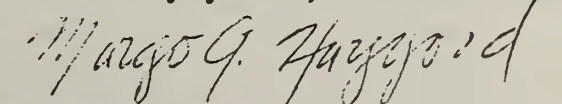
It is not clear to me that peer group review of hazards and safe guards adds very much to the initial assessment of the in-

vestigator. Although peer group review has the advantage of being ostensibly free from direct ties with the investigator, there are disturbing elements in this process. It provides that determinations of adequacy of safeguards will be made by other investigators doing similar work, whose outlook is likely to be similar to that of the investigator. Consequently, the added scrutiny of the peer group may not result in any substantive additional review. The justification for bypassing wider input is most likely that determination of hazards and adequate containment is liable to be highly technical, and much more readily made by those already familiar with the literature and procedures in practical terms. It would perhaps be advisable for peer groups reviewing such grants to appoint an auxiliary group, representing viewpoints outside their field, and outside the scientific community per se, to provide independent review of hazards and safeguards. Such a group could include persons with interests and background in laboratory safety, environmental safety, ecology, public health, law and citizens at large. The group would have to be educated in technical aspects of the problem, and would need to be committed to keeping up with developments in the field. Such a system might provide broader input, and avoid the limited outlook of the peer group.

Further the Program Advisory Committee should be diversified. Although I was most impressed with the sincerity and disinterestedness of the individual committee members that I met at the meeting, I believe that, as a matter of principle, conflict of interest, in reality or appearance, should be avoided. As Dr. Brown pointed out, expertise is always available in the form of expert testimony. Care should be taken to ensure that no one group is in the majority on the committee. Membership should be similar to that described for the auxiliary committee described above, including those directly involved in the research, those in fields indirectly bearing on questions of hazard, and some members outside the scientific community per se.

I will be very interested to see how you resolve this problem - one in which very little seems clear-cut. I appreciate the opportunity to participate in what I consider to be an inspiring precedent for science, and I hope my comments are useful to you in your decisions.

Sincerely yours,



Margo G. Haygood
35 Elm St.
Somerville, Mass.
02143

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG

Jack, Lulu and Sam Willson
Professor of Biochemistry

February 17, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Fredrickson,

First I want to express my deep appreciation for the invitation to participate in the meeting of your Advisory Committee on the matter of Recombinant DNA Guidelines; second, there is my sincere admiration for your wisdom in calling this meeting and for the efficient and extremely fair manner in which it was conducted. Without your skillful direction or a less sympathetic concern that all points of view be heard, the meeting would have been a useless exercise.

But now the responsibility for decision rests with you. Do we proceed with this research and under what conditions? This same question confronted the NAS Committee on Recombinant DNA Molecules at its meeting in April of 1974, and then challenged the participants of the Asilomar Conference on Recombinant DNA Molecules when they met just a year ago. Though the question can be put simply, the answer is far more complex. The choices, it seems to me, are limited. First, recombinant DNA research could be banned or postponed in all laboratories or institutions supported by U.S. government funds presumably to eliminate or postpone the hypothesized risks; but that action would cost us dearly in scientific progress at one of the most important and potentially rewarding frontiers of biomedical research. And, since other nations might not adopt that course, the protection we would be seeking could be ephemeral. Second, research with recombinant DNA molecules could be unleashed and thereby promote as vigorously as we could the attainment of the potential benefits from this line of investigation; the risks could be advertised, recommendations for their containment could be provided and the expectation for compliance left to the conscience of the investigator as well as the self-interest of the supporting institutions. Both solutions, I believe, are unacceptable to the large majority of the scientific community, the public and its representatives in government. The third choice is to proceed with this research cautiously, under a yellow light if you will, feeling our way and learning as we go, always prepared to step back if the light flickers red but ready to advance vigorously where the signals flash green.

The third option was the clear choice of the NAS Advisory Committee on Recombinant DNA Research and was endorsed nearly unanimously

Dr. Donald S. Fredrickson
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by the participants of the Asilomar Conference. More specifically, both recommended that certain experiments, though justifiable on scientific grounds, should be banned for the present. It was recommended that the bulk of possible experiments should proceed provided that the risk to the investigators, the public and the environment was minimal. I believe now, as I did earlier, that this is a prudent and defensible course to follow. The guidelines you are considering are stringent, perhaps more stringent than they need be on the basis of any evidence (not speculation) now available, but they will permit some experiments to go forward (in slow gear I should add). Many experiments, some argue the most important ones, are not possible and will have to await the development of certified safer means of physical and biological containment. But it is important that experiments get underway for only by experiments can we obtain the information needed to assess the benefits and risks of this methodology and to arrive at rational decisions in the future. Benjamin Franklin said it best when he addressed the Constitutional Convention during the Spring of 1786, in Philadelphia: "We are, I think, in the right road of improvement, if we are making experiments".

I believe that the guidelines take cognizance of many of the imaginable risks (perhaps even some of the unimaginable ones as well) and insist upon quite reasonable steps to contain these risks. The guidelines are intended to carry us through the short term and not to provide statutory law. There are undoubtedly some omissions and, depending on one's views, the constraints on the experiments and experimenters may be too lax or too stringent. But any flaws, if they exist, are more likely to be discovered by experience rather than in anticipation and these can be rectified quickly as there is clear provision for periodic review and revision of the guidelines. I cannot believe we are able now to anticipate every breakthrough, mistake, misrepresentation or flagrant violation. But we can be vigilant and committed to act promptly by tightening or relaxing the requirements and recommendations where there is a clear and undeniable risk to public health and safety. Similarly as risks and concerns are eliminated, there should be a relaxation in the need for highly contained systems.

If you detect a sense of urgency and some impatience in my views, that is intentional. I believe we are entering a period of explosive growth in our understanding of the genetic chemistry and function in all living things and very likely that knowledge will have revolutionary consequences on the biomedical sciences if not on most facets of human existence. Some have predicted that this methodology will allow us to capitalize on the unparalleled discoveries of the structure and workings of DNA and to use that

information to bring about practical benefits to human health and welfare. I agree with that view and because I am one who has been frustrated by the inadequacy of present day approaches, I reject as absurd the argument that we already can do safely what this methodology promises with its attending risks. It is pure unadulterated sophistry to argue that a five or ten year delay in achieving the promised advances is acceptable in the long term. On several occasions I've been asked why we are so eager to race ahead in our research. In making the transition from studying prokaryotes to the inordinately more complex eukaryotes, I, and most of the scientists who made that shift, have found progress tediously slow and unsure; compared to the achievements made with the simpler organisms, our present rate of progress is a snail's pace, primarily because we lack the means to dissect and analyze the more complex genetic structures and their functions. The recombinant DNA methodology offers a means to begin that task and encourages my belief that now we can make headway on what was becoming an intractable problem. To be able to dissect chromosomes from plants, animals and humans and to learn the secrets of their organization, expression and regulation promises great rewards to basic science and enormous benefits to medicine, agriculture and industry.

To argue further for urgency and not procrastination, let me point out that the world faces an impending shortage of insulin and that diabetics will be severely tried unless new sources of insulin are developed or more efficient use of existing supplies can be devised. If this line of research has even a chance of solving that problem, shouldn't we be promoting that advance? And what of the opportunity to develop more efficient sources or means of delivering of ammonia fertilizers for our and the world's crop needs? Can that wait five or ten years as well? Freeman Dyson recently cited the hidden costs of saying No!; he argued, cogently I believe, that it is as important to promote the class of experiments carrying no visible danger but still promising to unravel the complexities of genetic structures as it is to discourage experiments carrying danger to life on earth.

Before ending there are several features in the current version of the guidelines upon which I want to comment:

1. First there is the question of whether Escherichia coli is the most suitable (safe) host organism for molecular cloning experiments. No one disputes that E. coli is the best understood living organism on our planet. Because we are so familiar with its metabolism and genetic systems, E. coli provides us the best opportunity to achieve the benefits of this research and it is the most likely candidate for providing the safest host and vector systems. If we embark on a search for another, as yet unknown, but putatively more suitable, organism, what attributes should it have? As Roy Curtiss pointed out, it would have to have the very same type of genetic systems that make E. coli the risk. If it didn't inhabit man, what other ecological niche would it endanger? The search could take us ten,

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Page Four

twenty or more years and would we be more secure? I submit that any bright graduate student could construct ominous and catastrophic scenarios with any known organism. Therefore, I believe quite strongly that the suggestion to defer recombinant DNA research until an entirely new host-vector system is developed will confront us with the same dilemma we face now and I challenge any group of scientists to provide evidence to the contrary.

2. The guidelines as they are now worded are probably too inflexible; moreover, they are unnecessarily vague on how the flexibility the Committee desired could be achieved. For example, at La Jolla the Committee agreed that study sections should be able to take into account novel research objectives and experimental designs in interpreting and implementing the guidelines. Already there are innovations in experimental procedures that provide novel kinds of containment over and above that mandated in the guidelines; where increased safety can be documented, I believe it is appropriate to permit scientific review bodies to allow changes in the type of conventional physical containment or even the stringency of the biological containment. These should be ad hoc, carefully considered and any such rulings and their bases should become part of the body of knowledge that can guide others. Any procedure that frustrates scientific genius and its innovations will thwart rather than promote progress towards achieving greater safety. Iron-clad regulations or complex licensing requirements that can be changed only by acts of Congress would, I believe, be destructive rather than helpful. The only regulatory model I can now accept for monitoring or regulating recombinant DNA research in all possible settings is the one used for radioisotopes. Their sale, distribution, use and disposal are regulated for use in high schools, research labs, industry etc. Local committees, mandated and regulated by state and federal law, play a key role in monitoring compliance with safety regulations.

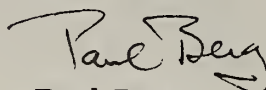
3. The function of local biohazard committees may also need some review. The present recommendation was intended to minimize the need for expert scientific peer review at each and every institution where this work would be carried on. Thus the suggestion that only certification of the physical containment should be considered locally. But I can see that in many institutions there is the scientific competence to advise investigators on scientific matters, e.g., experimental design, choice of host and vectors, assessment of risks etc. Such advice should not be admonished or discouraged when it is available; instead investigators should be encouraged to seek such advice from their local committees.

Let me say once again how pleased I was for the opportunity to participate in the deliberations of your Advisory Committee's hearings.

Dr. Donald S. Fredrickson
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The matter is in your hands and I have confidence it will be a wise and just decision.

Very sincerely yours,

A handwritten signature in cursive script that reads "Paul Berg". The signature is fluid and elegant, with a long horizontal stroke at the end.

Paul Berg
Professor of Biochemistry

PB:af

INSTITUTE OF SOCIETY, ETHICS AND THE LIFE SCIENCES

Hastings Center

DANIEL CALLAHAN
Director

February 18, 1976

Dr. Donald Frederickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Don:

Just a note to say how much I admire the very fine way in which you ran the meeting on the guidelines. You handled what could have been a very difficult situation just beautifully. As one who has to run meetings frequently, I was taking notes on the style of running meetings as well as on the substance of what was being discussed. Moreover, I felt that, by even calling the meeting, you did something historically significant. Like many others there, I felt that whatever you finally decide to do would represent a wise choice.

Sincerely,



Daniel Callahan

DC/em



New York State College of Agriculture and Life Sciences
a Statutory College of the State University
Cornell University

Department of Plant Pathology
334 Plant Science Building, Ithaca, N. Y. 14853

February 19, 1976

Dr. Donald S. Fredrickson, Director
National Institutes of Health
Building 1, Room 124
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

After reading the background material and listening to a 1 1/2 day presentation on the moral and public safety aspects of recombinant DNA research I have come to the conclusion that the rules promulgated by the NIH present a reasonable approach to this very complex and important problem. I personally have reservations concerning the risk to benefit ratio of this type of research and thus I feel that a go-slow, conservative attitude is warranted; to my mind the proposed guidelines reflect this concern. It is obvious too, that this is research which is world-wide in scope; rules established in this country will undoubtedly be used as a guide elsewhere to establish similar restrictions.

I would like to make a few specific comments about certain forms of research with higher plants, which might eventually enter into these studies. As treated in the guidelines, plants have only a peripheral role, but with a few significant advances they could well become very important in recombinant DNA studies. I'm not referring to the cloning of higher plant DNA and incorporating such DNA into bacteria; that eventuality is well covered by the guidelines. Rather, I'm concerned with the incorporation of foreign DNA into the genome of higher plants. At the present time this is just a dream, as the experimental work in this area has yet to demonstrate conclusively the stable incorporation of foreign DNA into the genome of higher plants.

As a vehicle for introducing foreign DNA, plant viruses might well play a role. Unfortunately (for these studies) most plant viruses contain RNA rather than DNA. There is however, one small group of five DNA plant viruses. These viruses are normally transmitted by aphids, but may also be transferred by mechanical inoculation. It is the property of aphid transmissibility which gives me some reservations as to the applicability of the guidelines. The physical containment guidelines are designed to prevent bacteria from getting out of the facility; thus, negative air pressures are specified, and the guidelines were merely extended to include plant studies. Negative pressures are certainly satisfactory for most plant studies except in those cases where insect-borne plant viruses are a part of the experimental procedure. Here tightly screened greenhouse facilities with positive air pressures plus antechamber entry rooms are normally used to avoid drawing insects into the greenhouse. Routine fumigations are also employed to keep the facility insect-free.

Dr. Donald S. Fredrickson

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One other aspect of plant work should also be incorporated into the guidelines in my opinion. That relates to containment of newly derived plant species, giving them adequate testing before allowing them to be planted in the field. The guidelines are quite adequate for the containment of plant cells, organelles and plant parts. Containment presents no problem here as these have low replication rates, fastidious nutrient requirements, don't form aerosols, etc. My concern relates to the lack of guidance once a new plant is produced. We need procedures to ascertain if, in addition to the introduction of desirable characteristics, we haven't inadvertently created an organism with undesirable characteristics.

I feel therefore, that the guidelines should include restrictions on the release of plants from controlled greenhouse or growth chamber facilities until they could be properly tested through several generations while the plant is still contained. Once the plant is propagated in the field it may be too late. Two areas where testing is warranted come to mind:

1). Plants and plant parts such as seeds and fruits should be tested to ensure that they are not toxic to animals which might be likely to eat them.

2). In the hoped for situation where the capacity for nitrogen fixation is introduced into new plant species testing should be done to ensure that these plants don't have a significant competitive advantage over other plants and thus become pests in themselves. We must assure ourselves that we don't produce a "super crab grass"!

Sincerely yours,



Milton Zaitlin
Professor

MZ/mh

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February 20, 1976

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Donald S. Fredrickson, M.D.
Director
National Institutes of Health
Building 1, Room 124
9000 Rockville Pike
Bethesda, Maryland 20014

Dear Don:

This letter is in response to your request that each of the members of the Ad Hoc Advisory Committee which met with you on February 9-10 to consider the proposed guidelines for research on recombinant DNA molecules furnish you with specific conclusions and recommendations. Although most of the comments set out below will repeat observations I made during the two days of the meeting, and particularly in my remarks at the end of the meeting, I thought it might be helpful to summarize them in one place.

I

From all the materials I have read on this subject and all that I heard at the meeting, there is no doubt in my mind that it is in the best interests of the public to proceed at this time with scientific research on recombinant DNA molecules. Although risks unquestionably exist, they are in no way insurmountable and, in any event, with appropriate control appear to be substantially less than the benefits to be obtained. Thus, the critical need is to devise control mechanisms to reduce the risks to a level that is low enough to be acceptable to the public, without at the same time raising unnecessary or onerous barriers to continuation of research in this field. I am confident that this balance can be struck.

Donald S. Fredrickson, M.D.
February 20, 1976
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As I stated at the conclusion of the meeting, the scientific community in general, and Dr. Berg in particular, deserve enormous credit for raising these difficult issues and dealing publically with them. Having taken this initiative, I believe the scientific community should be permitted to keep it, as long as it continues to pursue a reasonable means of protecting the public against unnecessarily large risks from this experimentation. If this objective is actively pursued and achieved, I believe it unlikely that the public would seek additional legislation or other restrictions that might impede the scientific research involved. If it is not pursued at this time, however, I find it unlikely that the public will allow this type of research to remain uncontrolled, and additional legislation or other forms of restriction will in time undoubtedly be imposed. Moreover, the history of government regulation has shown, time and again, that when a group fails to exercise reasonable control over its activities which impinge upon the public the governmental controls that inevitably follow have imposed far more stringent restrictions than could initially have been utilized by that group on a voluntary basis. I would therefore urge that the scientific community turn its attention, as soon as possible, to devising the least restrictive form of control mechanism that will both reduce the risks to the smallest level that is reasonable and at the same time permit the continuation of this important research without crippling legitimate scientific inquiry by qualified experts.

II

The public is becoming far more sophisticated with respect to scientific issues than many in the scientific community recognize. There is wide understanding that "absolute safety" is not attainable and, in any event, is not proveable. Thus, the term "safety" is used by me and others, including Congress, to mean nothing more than a level of risk that is both reduced as far as is reasonably feasible under the circumstances and does not outweigh the potential benefits.

At the same time, the public clearly expects that the burden of showing an acceptably low risk is on those who propose to proceed with the project involved -- whether it be industry, academic scientists, or other groups. It is wholly insufficient, in my view, to argue that no harm has been proved; the burden is on the scientific community to prove to the public that the risks from research on recombinant DNA molecules have been reduced

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to the lowest level that can reasonably be achieved, that the level of risk to the public is extremely small, and that the potential benefits are substantial and are significantly greater than those risks. This principle has been embodied in all new public health and safety legislation for at least the past 20 years, and prevades public opinion today. It is therefore essential that the preamble to the final guidelines be expanded to discuss the potential risks and benefits at length, to satisfy this "burden of proof."

A number of the specific provisions in the guidelines raised substantial questions in my mind. Obviously, I am not technically trained and thus have no qualifications to determine the adequacy of or justification for many of these specific provisions. With respect to each of the provisions, however, it does seem clear to me that the preamble to the final guidelines must be expanded to deal directly with the issues that both non-scientists and scientists have raised. Again, this is one aspect of satisfying the burden on the scientific community to show to the public that a favorable benefit/risk ratio exists.

At the meeting, there were, of course, strong views expressed both that the guidelines are too conservative and are too liberal. It is not sufficient, as you know, simply to say that the present draft of the guidelines must be about right, since the criticism from the opposing sides appears equally balanced. The "middle ground" approach is often fallacious. What is needed, instead, is a reasoned analysis of the views expressed by all who attended the meeting, a statement of the resolution of the issues that arose, and a specific rationale for the resolution of each such issue. All points of view were well-represented at that meeting and there was full opportunity for them to be stated and explained. All speakers seemed extremely well-intentioned, serious, and dedicated both toward the advancement of science and adequate protection of the public. Their specific concerns clearly deserve close attention by you, in articulating the results of your decision.

Much of my probing during the meeting, as well as during the breaks, was designed to determine whether any aspect of the proposed guidelines was clearly unsupported or irrational. As I have already stated, it is not feasible for me to attempt to offer any specific technical recommendations on the scientific issues, beyond this very broad type of determination. On the basis of the material I have read and the discussion at the meeting, there are only a very few areas where I can offer specific

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comments. The remaining portions of the guidelines appear justified, with the qualification that the rationale for those portions should, as I have discussed in this letter, be spelled out in much greater detail.

My specific comments and recommendations with respect to the proposed guidelines are as follows:

1. From all that I heard at the meeting, as well as my own background in the field of government regulation, I am satisfied that the concept of "physical containment" is both too imprecise and too subject to the vagaries of human fallibility that it does not offer sufficient protection against harm except for those experiments for which the risk is so extraordinarily low that it is virtually non-existent. For any experiment in which there is a significant risk to the public, therefore, development of "biological containment" safeguards is absolutely essential. If you wish to have specific documentation for the fallibility of physical containment, I would suggest that you review the Food and Drug Administration experience with compliance by the regulated industry with good manufacturing practices, compliance by investigators with requirements such as those for obtaining informed consent, and compliance by all types of those laboratories with acceptable animal testing procedures.

Accordingly, NIH should place a very high priority on exploring the precise level of risk involved in the various experiments on recombinant DNA molecules that have been or may be undertaken, and in developing and confirming biological containment both for E. Coli (EK-2 and EK-3) and for other organisms to be used as hosts and vectors. Serious consideration must be given to the possibility of prohibiting use of EK-1 and other organisms which do not reach an acceptable biological containment level after an interim period of time, as suggested by the Boston group; and if you believe that this is not necessary in order to protect the public you should clearly state the basis for your decision.

2. Prior to the meeting, it appeared that the Boston group was making a major argument that E. Coli simply should not be used at all as the host organism. The argument made in their prior submissions was based on the concern that, since E. Coli is a normal resident of man, it simply is not a safe host for this experimentation. At the meeting, on the other hand, the representatives of the Boston group appeared to me to recede from this position, and to accept the argument that development of EK-2 and EK-3 organisms would provide for sufficient public protection.

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Nonetheless, I believe it would be useful for the preamble fully to discuss this issue and to state why development of EK-2 and EK-3 organisms will provide adequate public protection even though E. Coli is prevalent in man.

3. I would urge reconsideration of the use of SV-40 virus with anything less than a physical containment level of P-4 at this time. The written materials I have seen contain no scientific documentation for the assertion that this cancer virus, which clearly can infect man, produces no harmful effects. Indeed, although this assertion was made by two individuals at the meeting, in discussions during breaks in the meeting a number of people hotly disputed that assertion. Some have stated that there is clear evidence that the SV-40 contamination of polio vaccine did in fact result in harmful effects in children.

I am not technically competent to judge the merits of this issue. On the other hand, any scientist who wishes to use in this type of experimentation a virus which is known to cause cancer in animals, and which is also known to infect humans, clearly must bear a substantial burden of showing that there is no possibility whatever of harm to man before it should be permitted. Any doubt whatever should be resolved on the side of public safety. At the very least, therefore, if you do not conclude to ban all experimentation with SV-40 or at least place it under P-4 conditions, the preamble to the document must explain in detail the reasons for your decision.

4. The guidelines should be carefully reviewed to determine proper use of the terms "shall" and "should". To a lawyer, "should" means "you are not required to do this." As Dr. Hogness stated at the meeting, the NIH Advisory Committee paid little or no attention to this distinction, and it is therefore necessary that this aspect of the guidelines be reconsidered in detail.

5. The guidelines should also be revised to state explicitly what will be done with existing cloned material of a type that will no longer be permitted. Recommendations with respect to this issue were included in the minutes of the NIH Advisory Committee's meetings, but were not included in the guidelines themselves.

6. The procedure for approval of EK-2 and EK-3 materials should be stated explicitly in the guidelines. The guidelines should also state that no experiments may be conducted with EK-2 and EK-3 organisms without first obtaining approval of the NIH Advisory Committee. These conclusions were apparently also included in the minutes but not fully reflected in the guidelines.

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7. I am uncertain whether other concepts were also included in the minutes of the drafting committee but were not incorporated into the guidelines themselves. The guidelines should be a self-contained document; the minutes have no official status. I would suggest that the minutes be reviewed in detail to make certain that additional material was not lost in this manner.

8. I believe it would be helpful to state explicitly both that all grant and contract requests must contain a full analysis of the benefit/risk issues involved in the proposed experiments, and that this analysis will be made publically available.

9. Public participation in decisions with respect to these experiments should be substantially strengthened. Unfortunately, there was no discussion of this specific matter during the meeting, and thus it is not feasible to offer concrete suggestions with assurance that they are practicable. It appeared to me that public representatives should be added to the NIH Advisory Committee which will revise and implement these guidelines (e.g., people with the sensitivity and stature of Mrs. Esther Peterson) and that public representatives should also sit on the local biohazard committees. It was also my impression that the function of the local biohazard committees should be substantially broadened, in the way that the functions of peer review committees for drug investigations and similar activities have been broadened in the past few years. On the other hand, Dr. Hogness raised with me after the meeting some potential practical difficulties with some of these suggestions, and I realize that further discussion might well lead to other means of obtaining this same objective. My principal concern is that this objective should be addressed and implemented in some way.

10. Substantial reconsideration of the P-1 through P-3 requirements should be undertaken. One possibility would be to collapse P-1 and P-2 into a single strengthened level of physical containment. A second possibility would be to upgrade some of the experiments to a P-3 or P-4 level until greater experience is obtained with them.

In any event, it is quite clear to me that the present provisions in the guidelines do not adequately describe the intended requirements for P-1 through P-3. The experience of the Food and Drug Administration in issuing similar requirements for "good manufacturing practices" has been that general require-

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ments are inadequate to assure appropriate activity. The more specific the requirement, the greater will be the adherence to it.

I was struck, for example, by two points raised by Dr. Barkley in his slide presentation. He pointed out that an ordinary kitchen blender should not be used but, if it is used, the lid should be kept on it for ten minutes after use. He also said that a P-3 area should not have access to a well-travelled corridor. I then looked in the guidelines and could find neither of these concepts stated as requirements. Nor is there anything in the guidelines which states the minimum level of scientific training and experience necessary to conduct any of these experiments (e.g., either the number of years of work in the field or the level of education received). Some reference was made at the meeting to other NIH publications which discuss some, but apparently not all, of these matters, but those other publications are not even referenced in the guidelines, much less incorporated as requirements. It appears to me either that those other publications should be incorporated as requirements, and perhaps supplemented by additional requirements in the guidelines, or all of these detailed requirements should be spelled out in the guidelines themselves. It simply is not adequate to refer to acceptable practices, with the vaguest of generalized descriptions, since this is tantamount to no requirement whatever.

11. A number of people pointed out that the concept of "99% purity" should be fully defined, and the guidelines should either contain a scientific procedure for determining this level of purity or require approval of such material by the NIH Advisory Committee.

III

The procedural status of the guidelines raises both legal and policy issues.

The initial purpose of the guidelines is to determine the circumstances under which NIH will fund recombinant DNA molecule experimentation. Obviously, NIH intends to enforce the guidelines by granting or denying funds. Under these circumstances, it seems disingenuous to call the document "guidelines." It is intended to be rigidly enforced by NIH, and therefore has the same legal status as a regulation, rule, or even a statute.

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I recognize that the scientific community does not like the term "regulation", and indeed that NIH abhors the idea that it might in any way be an "enforcement agency." If it is useful from a psychological standpoint to preserve this fiction, I see no objection to continuing to call these requirements "guidelines." At the same time, however, no one should be misled into believing that they will not be rigidly and completely enforced, through the grant and denial of NIH funds.

From a legal standpoint, I believe there is a substantial possibility that a court would determine that these guidelines may not be enforced by NIH even for funding purposes unless they are proposed for comment and adopted through publication in the Federal Register. Emerging case law indicates that requirements of this type cannot be adopted without the public procedures required by the Administrative Procedure Act, through Federal Register publication.

Wholly apart from legal considerations, from the standpoint of sound public policy I believe that these guidelines should be published in the Federal Register for comment, and then republished in final form on the basis of all comments received. Everyone has acknowledged the courage and integrity of the scientists who have raised this matter as a public debate. I believe that this process should be continued to its logical conclusion, i.e., publication in the Federal Register. Any possible criticism that the issue has been debated only by scientists, and not by the public at large, would then be laid to rest forever.

Publication in the Federal Register for general comment by the public would not mean that resolution of this matter, and the pursuit of important scientific research, would be delayed for yet another long period of time. On many occasions, proposed regulations have been implemented on an interim basis pending receipt of comment and publication of final regulations. I see no legal reason why the guidelines could not be implemented, for purposes of NIH funding, pending completion of the public procedures established in the Administrative Procedure Act.

Nor does publication of the guidelines as regulations in the Federal Register mean that the guidelines will forever be chiseled in stone as rigid requirements that cannot be changed as new scientific information becomes available. Two mechanisms are available to assure flexibility in regulations embodying complex scientific requirements.

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First, and most obvious, amendments can be proposed to regulations, and can be expedited, on the basis of any new information which justifies a change in the existing regulations. This does take time, but it is not as difficult a procedure as many believe. Second, and perhaps more to the point, the regulations themselves can incorporate a procedure for the grant, by the NIH Advisory Committee, of variances from the regulations on either an ad hoc or a class basis. Two examples of variance procedures presently incorporated in Food and Drug Administration regulations may be found in 21 C.F.R. 10.5 (temporary permits to vary from a standard of identity for food) and 21 C.F.R. 1010.4 (variances from performance standards for electronic products). Such variances permit instant flexibility, while at the same time preserving adequate public protection. Thus, publication of the guidelines as regulations need not in any way interfere with the flexibility that is necessary for the rapid advancement of science in this area.

Adoption of these guidelines as regulations would, in my judgment, have a substantial beneficial impact. It is important to convince the public in general, and Congress in particular, of the legitimacy of the entire mechanism by which NIH and the scientific community are pursuing this matter. Subjecting the entire process to close public scrutiny through publication in the Federal Register would, I believe, accomplish this purpose, and perhaps alleviate many of the concerns which have been expressed about recombinant DNA molecule experimentation.

IV

Adoption of final NIH guidelines does not appear to me to end this matter. NIH guidelines, whether or not they are published and adopted in the Federal Register, are enforceable only through the NIH grant and contract mechanisms. The guidelines may also carry the force of moral suasion for the scientific community as a whole, but anyone who is not conducting recombinant DNA molecule experimentation with NIH funds would be under no legal obligation whatever to follow these guidelines. Thus, experimentation funded by charitable foundations, state and local agencies, public school systems, private educational institutions, industry, and any other private individuals or institutions, would be free to conduct whatever experimentation on recombinant DNA molecules it may conclude to pursue.

I believe it extremely unlikely that Congress and the American public will be willing to rely solely on the moral suasion engendered by these guidelines, and the peer pressure that they

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will carry, to determine that no experimentation will be undertaken in the country in violation of the guidelines. Proliferation of experimentation in violation of the guidelines is, in short, a legitimate public concern. The concern is not simply that a single scientist or his co-workers will be harmed, but rather that an accident will result in an epidemic with major public harm. Moreover, it is not unlikely that any violation of the guidelines would occur not in those institutions which have the greatest background and experience in proper experimentation, but rather in those institutions where knowledge of this type of work, and the hazards it may cause, is only rudimentary.

I therefore believe that it is imperative for NIH and the scientific community immediately to consider various other regulatory possibilities for controlling this type of experimentation by assuring adherence to the guidelines by all segments of the scientific community, including those not funded by NIH. Various possible regulatory models are, of course, available. I am not confident that I presently understand enough about this area of scientific endeavor to be able to state with assurance at this time exactly which form of regulation would be sufficient to assure adherence to the guidelines yet impose only the minimum level of regulatory control necessary to achieve the intended purpose. I am concerned, however, that if the scientific community does not face this issue, it will soon have imposed upon it far more stringent and onerous regulatory requirements than are necessary and appropriate under the present circumstances.

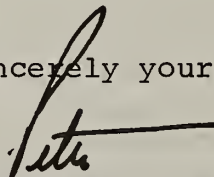
The most extreme possibility of regulatory control would be a requirement that the Federal government approve every recombinant DNA molecule experiment before it is conducted. I believe this type of rigid and complete control is wholly unnecessary, and should not be adopted. Far less stringent controls would appear to be sufficient to assure public protection. One possibility would be to license all laboratories and/or researchers conducting this type of experimentation. A second possibility would be to license the materials used in these experiments, such as the enzyme and the source of the foreign DNA, when they are intended for this specific use. Either or both of these possibilities could be accomplished without any change in existing legislation, under the provisions of Section 361 of the Public Health Service Act, 42 U.S.C. 264, which authorizes the Secretary of HEW to promulgate regulations necessary to prevent the introduction or spread of communicable disease. Another possible means of regulatory control would be to impose these guidelines on all

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federal agencies and all institutions and entities which receive any form of federal funds, by a presidential executive order. I am confident that, if the matter were pursued further, other alternatives would also come to light.

My concern is only that this matter should be addressed as soon as possible. Having taken the initiative in handling this matter thus far, I believe the scientific community should pursue it vigorously in order not to run the risk that Congress will conclude that it is necessary to enact stringent new regulatory controls covering this type of experimentation in order adequately to protect the public.

Sincerely yours,

A handwritten signature in dark ink, appearing to read "P. Hutt", with a long horizontal stroke extending to the right.

Peter Barton Hutt



DEPARTMENT OF BACTERIOLOGY
AND IMMUNOLOGY

BERKELEY, CALIFORNIA 94720

February 23, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Frederickson:

I want to congratulate you on the quality of the DNA Recombinant meeting. The background science and the rationale for the proposed guidelines were clearly explained, and the insiders and outsiders in the field were given every opportunity to express their opinions. The result was an amazingly good-willed and serious concentration on the real issues.

In my opinion these issues cannot be resolved in a permanent way at present because the available data do not allow adequate evaluation of the relative potential for benefit and harm. (Phil Handler expressed it well when he commented that he had listened to all speakers and agreed with each one). On the other hand, controls are needed at present for the pursuit of the critical data and for the prevention of underground uncontrolled experimentation. On this basis it seems to me paramount that:

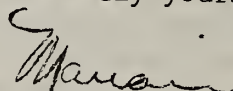
1. A set of rules representing the best bets of the moment be adopted as temporary working orders. These should certainly not be processed as formal regulations and they could include a self-destruct clause by mandating review and revision every year. Nor should the first set of regulations be too rigid since they could result in oversell to the nonscientific community and prevent the possibility of future relaxing modifications.
2. The NIH consider making on a crash basis facilities for pioneering experiments which will clarify the dangers and difficulties. These might even be experiments not allowed under the present rules, but their aims must clearly be to provide the information for better rules or provide the safe "organisms" and procedures which can be used in the field. These facilities might properly be at Fort Detrick.

Dr. Donald S. Fredrickson
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3. The NIH consider making a few P4 containment facilities for workers throughout the country which would be available on a shared basis like the Brookhaven accelerator or the New England Enzyme Center. This would mean that young investigators or individuals at less favored institutions could do experiments and it would eliminate the serious problem of favoritism that will inevitably arise from the award of facilities to a few institutions.

I hope these comments are helpful to you and I wish you good decision-making.

Sincerely yours,



Marian E. Koshland
Professor

MEK:kg

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3-4700

February 25, 1976

Dr. Donald Frederickson
Director
National Institute of Health
Bethesda, Maryland 20014

Dear Dr. Frederickson:

Many of us who work in biological laboratories are concerned about particular weaknesses in the new NIH Biohazards Guidelines. We approve of the concept of institutional safety committees. However, we worry that such committees will be appointed, rather than elected, and that they will be composed solely of faculty and research directors. Since all people who work in and around laboratories will be subject to risk, and since only those who actually do the work have direct knowledge of the procedures followed, we feel that safety committees should be composed of representatives of all strata of the research workforce: technicians, graduate students, glassware washers, supervisors, postdoctoral fellows, custodians and clerical personnel.

Here at M.I.T. we have organized such a committee. One of our first efforts was distributing a questionnaire on safety procedures to all people working in the building. The results were deeply disturbing. Only a very small minority had received safety instruction, regular medical examinations, or safety updates. At present safety is left in the hands of research directors; since they have a strong vested interest in the carrying out of research at maximal speed, safety issues are often perceived by them as simply an impediment to research.

We encourage you to insist that the guidelines clearly state that the safety committees must be representative of all segments of the workforce at risk.

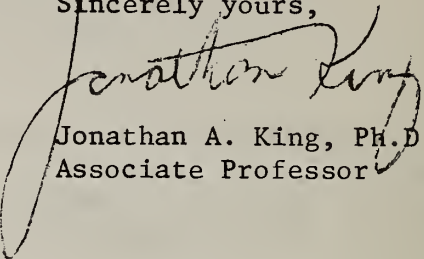
Our own experience has also taught us that safety and safety committees require money. We are not sure how this should be provided; whether as a % of overhead of each grant, or with the NIH providing funds to match institutional funds; or whether institutions be required to provide safety budgets as a precondition for obtaining grants. However, we feel that this aspect of the issue is a high priority.

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Letter for Jonathan King, M.I.T.
concerning Health and Safety

The safety of people who work here has to be put first. The ultimate role of the biomedical research effort is to improve the national health, lets start right at the beginning with the health of those who work at the research. Sad indeed if we can spend millions to help unknown individuals in the distant future, while the health and safety of those with us at present is neglected.

Sincerely yours,

A handwritten signature in cursive script, appearing to read "Jonathan King". The signature is written in dark ink and is positioned over the typed name and title.

Jonathan A. King, Ph.D.
Associate Professor

JAK:rm

Committee:

Camille Horkun
Josie Calareso
Allen Silverstone
Bob Ludwig

6724 Towne Lane Court
McLean, Virginia 22101
February 27, 1976

Dr. DeWitt Stetten, Jr.
Deputy Director for Science
Building 1, Room 122
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Stetten:

I am writing in reference to the proposed guidelines for research involving recombinant DNA molecules. Although I am the liaison officer from the National Academy of Sciences to the Recombinant DNA Molecule Program Advisory Committee of the National Institutes of Health and was present for most of the meeting in La Jolla in early December 1975, when the guidelines were drafted, several points occurred to me only during the discussions that took place on February 9-10, 1976, at the special meeting of the Advisory Committee to the Director of the NIH.

1. On February 9, 1976, at the meeting of the Advisory Committee to the Director of the NIH, Dr. Robert G. Petersdorf discussed Escherichia coli as an infectious agent. He stated that E. coli almost never causes disease in man, and that manipulation such as catheterization is necessary for urinary tract infection with this organism to take place. Although I realize that the problem is not so much one of the pathogenicity or virulence of E. coli per se, but of the hazards of using E. coli as a carrier of genetic information, particularly in its plasmids, I would like to add to what Dr. Petersdorf said, which, since it applied only to E. coli infection in adult males, leaves the inaccurate impression that E. coli is harmless.

Infections of the urinary tract are common in the female at any age, but more so during pregnancy. E. coli is one of the most common etiologic agents of urinary tract infection. The extent of E. coli K-12 survival in the human urinary tract is not known, and it may not be possible to know. But limited survival of K-12 in the human gastrointestinal tract has been documented. Such survival would permit migration to the female urogenital organs. From what is known young women are at higher risk than others of infection by E. coli. Most laboratory technicians are women in the peak of their reproductive years. One person raised the issue of informed consent with respect to persons working with recombinant DNA molecules and suggested that only male technicians be hired. This fails to make the point that if indeed there is a risk in working with E. coli, young women in their reproductive years are at even greater risk, because of their normal susceptibility to infections of the urinary tract and because they

are at risk for pregnancy, which further increases such susceptibility; these women should be appropriately informed prior to employment. Maternal infection might also place the fetus at risk for teratogenic effects from recombinant DNA carried by E. coli. In addition, in the first month of life, gram-negative bacilli of enteric origin are the most common cause of bacterial meningitis. This disease, often acquired during birth, has a high mortality rate and a high permanent disability rate.

The National Institute of Allergy and Infectious Diseases is also concerned about diarrhea, meningitis, and urinary tract infections caused by E. coli (See attached agenda of meeting on Current Status and Prospects for Improved and New Bacterial Vaccines).

In summary, there is no information on the ability of E. coli to colonize the female urinary tract. There is also no information on what effects viral or plasmid DNA might have on a developing fetus. The main point is that E. coli is an opportunistic pathogen and has been demonstrated to cause severe illness in infants and in young women. Recombinant DNA carried by E. coli might also pose a risk for the developing fetus.

2. My second area of concern is the use of SV-40 virus DNA. The effects of slow viruses on the developing brain are just now coming under study. What effect these viruses, which probably include the SV-40 viruses, may have on the production of disorders of the nervous system is not known, but many human neurological diseases are now known to be related to chronic viral infections. During the February 9-10 meeting, several speakers stated that, although SV-40 virus was inoculated into hundreds of thousands of children along with the Salk vaccine, no human illness has resulted. Two papers have been published on the effects of SV-40 contaminated vaccine in man. M. D. Innis ("Oncogenesis and Poliomyelitis Vaccine" Nature 219:972, 1968), examined the oncogenic potential of parenterally administered poliomyelitis vaccine contaminated with SV-40. This study was done in Australia, where careful immunization records are kept on all hospitalized children. A significant difference was found in malignant disease in children over 1 year of age between those who had been immunized against poliomyelitis and those who had not. D. M. Baguley and G. L. Glasgow ("Subacute sclerosing panencephalitis and Salk Vaccine" Lancet 2:763, 1973), reported the emergence of subacute sclerosing panencephalitis (SSPE) in New Zealand after September 1956, when the Salk vaccine was introduced. They feel that the Salk vaccine, and therefore SV-40 virus is implicated as a factor in the development of SSPE in New Zealand. Although again there is no direct evidence

implicating SV-40 as a causative agent of disease in man, not all the reports are negative, and follow-up data are as yet insufficient. Surveillance should be continued, because insufficient time may have elapsed since the administration of vaccine to be sure that no disease will result. The association of SV-40 virus with human tumors, such as metastatic melanomas and meningiomas, was referred to in the bibliography of Appendix B of the guidelines, but these papers were not referred to during the discussion at the February 9-10 meeting, leaving the misleading impression that no human disease has been associated with SV-40.

3. It became clear during Dr. Brown's presentation on February 10 that the guidelines preclude "shotgun" experiments with cold-blooded vertebrates until an EK2 host becomes available. Prior to February 10, my impression was that EK2 was available and did not need further certification by the NIH advisory Committee. Cold-blooded animals (invertebrates) and lower eukaryotes are now all in one class requiring P2 physical containment and EK1 host vector, unless the eukaryote in this class is a known pathogen or carries such an agent. Thus, "shotgun" experiments with any inveterbrate can be done now. The classification for "shotgun" experiments on the slide shown at the February meeting was simply "cold-blooded vertebrates" and the "lower eukaryotes". The "lower eukaryotes" include the arthropods. The phylum Arthropoda contains 85% of all known species of animals. In this phylum, the insect class is the largest and includes 800 species that have been described and probably missions that have yet to be described. The evolution of insects has at least three levels of specialization, and, according to C. P. Hickman (Biology of Invertebrates. C. V. Mosby Co., 1967, p. 449 ff), "Insects are the most extensive group of organisms within the animal kindgom and have reached a high peak of invertebrate evolution." Insects have evolved very rapidly, compared with other major groups, and have adapted to a wide variety of ecological niches. Thus, it would be difficult to include insects among "lower" eukaryotes. Appended Attachments 2 and 3, are two evolutionary charts showing the independent evolution of insects and vertebrates; insects are not "lower" than vertebrates; perhaps the guidelines should be modified, because the term "lower" is not clear regarding complexity of organization or of evolution. With respect to the possibility of a risk to man, "shotgun" experiments involving the DNA of insects are permitted with EK1. In K. G. V. Smith's, Insects and other Arthropods of Medical Importance (British Museum, London, 1973), a vector table lists insects and other arthropods involved in the transmission of organisms pathogenic to man. Pathogens carried by insects include bacteria, nematodes, protozoans, rickettsia, spirochetes, and viruses. Over 270 species of insects and arthropods are listed as vectors of more than one human pathogen. These known pathogens require higher containment. However, because of the multitude of insect species and of their symbiotic arrangements with microorganisms,* it is

* Many insects have symbionts, such as protozoans, which aid with the digestion of food.

difficult to isolate DNA from an insect without carrying along with it DNA from microorganisms; the hazard of such DNA to man and other organisms is unknown. For example, *Drosophila*, because of its plasticity in evolution and easy breeding, is one of the favorite organisms for genetic research. However, it is very difficult to separate *Drosophila* from yeast, and DNA extracted from *Drosophila* probably contains yeast DNA.

In addition, literature exists dating back more than 10 years on the transmission of mycoplasma-like symbionts as a hereditary infection in *Drosophila* species. These symbionts are transmitted via the egg cytoplasm and enter the future germinal tissue of adult flies. Further, spirochetes that infect *Drosophila* eggs and alter the sex ratio of offspring have been described. A DNA virus able to infect and to multiply in these spirochetes is also known. Active virus has been extracted from *Drosophila melanogaster*, as well as from other species. Thus, it is very difficult to separate *Drosophila* DNA from the DNA of microorganisms that might remain associated with it even after prolonged breeding in the laboratory.* These are only a few instances of known association between microbes and insects. These associations may or may not have significance for man, but the insect class is so vast and so varied that the guidelines should consider it. There seems to be no biologic rationale for considering insects to be safer than cold-blooded vertebrates. This point was raised at La Jolla by Dr. Brenner, but the Committee voted to keep a distinction between the cold-blooded vertebrates and other cold-blooded animals, including insects. Because the Committee did

* Sakaguchi, B. and D. F. Poulson. Interspecific transfer of the "sex-ratio" condition from *Drosophila willistoni* to *D. Melanogaster*. *Genetics* 48: 841, 1963.

Oishi, K. and D. F. Poulson. A virus associated with SR-spirochetes of *Drosophila nebulosa*. *PNAS* 67:1565, 1970.

Ehrman, L. and R. P. Kernaghan. Infectious heredity in *Drosophila paulistorum*. A Ciba Foundation Symposium, 25-27 January 1972. Pathogenic Mycoplasmas. Associated Scientific Publishers, Amsterdam.

Ehrman, L. and S. Daniels. Pole cells of *Drosophila paulistorum*: Embryologic differentiation with symbionts. *Australian J. Biol. Sci.* 28:133, 1975.

discuss the issue, I did not comment at that time; however, during the meeting on February 9-10, in listening to Dr. Hogness and Dr. Brown, I thought that a rationale for EK1 containment for insects should be put into the guidelines. If cold-blooded vertebrates are indeed felt to pose a hazard, the insects do not pose any less of a hazard in "shotgun" experiments. Thus, perhaps EK2 should be used for all insects as well, especially if Dr. Curtiss's progress in the development of an approved EK2 makes the safer organism available very shortly. In that case, there seems to be very little rationale for using an EK1 as a host vector for the insect class. Also, the use of embryonic or germ-line DNA was thought to be safer than other DNA from cold-blooded vertebrates. Dr. Margery Shaw in her concluding statement did not feel that embryonic material was necessarily "cleaner", although horizontal infection might be less likely. The examples quoted above of spirochetal and viral infection of *Drosophila* eggs represent an instance in which embryonic material is infected. There are undoubtedly many others.

4. My last point concerns genetically active DNA released by bacterial cells dying in an animal host. DNA has been demonstrated to be genetically active in the mouse. In fact, Griffith's 1928 experiments involved the transformation of living pneumococci by DNA released from dead pneumococci in mice. Austrian, in 1952, demonstrated transformation of pneumococci with DNA injected at a distant site in several species of mammals. Several other investigators have done similar experiments; thus, even though it might be expected that extracellular DNA would be extensively digested in animals, some DNA can resist degradation and retain genetic activity in vivo.

If there are risks in this type of research, the genetic activity of released DNA must be considered, not only the survival of the host microorganism.

* Austrian, R. Observations of the possible role of nucleic acid exchange reactions in pneumococcal capsular type transformation. Bulletin of the Johns Hopkins Hospital, 90:170, 1952.

Conant, J. E. and W. D. Sawyer. Transformation during mixed pneumococcal infection of mice. J. Bact. 93:1869, 1967.

Nightingale, E. O. Competence of pneumococcal isolates in bacterial transformations in man. Infection and Immunity. 6:785, 1972.

Dr. DeWitt Stetten, Jr.

February 27, 1976

Page 6

The concerns discussed in this letter and mentioned briefly during the meeting of the Advisory Committee to the Director of the NIH are expressed here solely as those of an individual scientist and physician. I thank you for the opportunity to attend the meeting of the Advisory Committee and the meeting of the Recombinant DNA Molecule Program Advisory Committee of the National Institutes of Health and to express my views. In spite of my concerns, I would like to state my whole-hearted endorsement of the general spirit and content of the guidelines, which I hope will be adopted very soon.

Sincerely,

Elena O. Nightingale

Elena O. Nightingale, M.D., Ph.D.

EON:LM

Attachments

The same letter was sent to Dr. Fredrickson

CAMPBELL CLINIC ASSOCIATION, INC.

525 MCCALLIE AVENUE
CHATTANOOGA, TENNESSEE 37402
(615) 265-3141

GENERAL SURGERY

GUY M. FRANCIS, M. D.
JOSEPH J. DODDS, M. D.

ADMINISTRATION

TED P. CROWLEY, ADMINISTRATOR
E. HOWARD WADDELL, ADM. ASST.
THAD C. DRENNAN, ADM. ASST.

ORTHOPEDIC SURGERY

EARL R. CAMPBELL, JR., M. D.

ANESTHESIA

RAYMOND S. TENENBAUM, M. D.

GENERAL PRACTICE

WALTER H. SMARTT, M.D.

INTERNAL MEDICINE

E. WAYNE GILLEY, M. D.
MERRILL F. NELSON, M. D.
WILLIAM C. DOWELL, M. D.

GYNECOLOGY

STANLEY J. DRESSLER, M. D.

February 27, 1976

Dr. Donald S. Fredrickson
Director, National Institutes of Health
Building 1, D.H.E.W.
Bethesda, Maryland 20014

Dear Dr. Fredrickson,

It was indeed a pleasure to participate in the recent discussions concerning the Bio-Hazards of DNA Research. It is my opinion that the N.I.H. is to be commended for the forthright approach it has taken concerning such a controversial issue and also for the dissemination of such information in a non-sensational manner. In a like manner, the scientific community, as a whole, has been very responsible in its action to delay activity until appropriate regulations can be developed.

In addition to the factors already reduced to writing in the proposed guidelines, I feel the following considerations must be addressed in any final set of regulations: (not necessarily in order of importance)

- 1) The introduction to the regulations must contain a positive statement that the potential inherent benefits of genetic engineering outweigh the potential risks and for this reason experimentation in the field should continue. In addition, there should be wording to indicate a mood of caution, lest the scientific community becomes apathetic and abdicates its control effectiveness. This is what I consider to be a privilege/responsibility ratio and must be recognized by all who conduct hazardous research activity.
- 2) The regulations must be explicit in definition and phrasiology. There must be a minimum latitude for individual interpretation. Words such as 'banned' must be carefully explained as to their meaning and all phrasiology must be mandatory as opposed to obligatory.
- 3) The National Institutes of Health should immediately undertake multifaceted experiments to secure the necessary data to allow for ongoing responsible decisions

concerning risks, the ability to destroy or contain altered genes, as well as encouraging the further development of safer vector/host systems.

4) A communication system must be established worldwide that will permit almost instantaneous alerting in the event of experimental misadventure, the intent being to prevent repetition, as well as to bring all minds to bear on developing a solution to the problem created.

5) All individuals involved in this variety of experimentation must understand the dangers of that involvement. Informed consent is essential, particularly as it relates to individuals of less than scientific background who through ignorance may not understand the gravity of their working conditions.

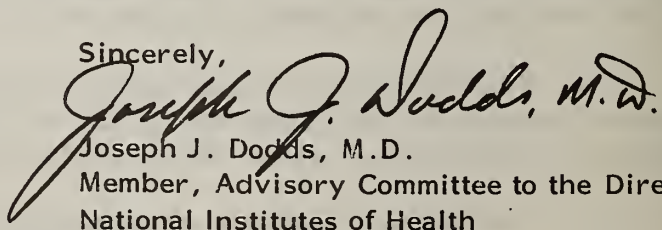
6) An appropriate Appellate Mechanism must be established to allow for individual requests for variances from established guidelines. The granting authority, however, must be broadly representative of knowledgeable peers in genetic engineering and any decision to grant a variance should be a unanimous opinion of all members of the granting council. Variances should apply only to a single individual and not an entire laboratory or system, and should be public knowledge to all investigators so that they are aware of who is doing what.

7) There must be an ascending pyramid of review and comment on policy, planning and implementation with both local and national involvement. The review must be on a consumer, as well as scientific level. It is essential that control of the scientific process remain within the fraternity of investigators and they must act always in the best interest of the public, less the Congress mandate that review authority be delegated elsewhere.

8) Probably the most important issue is that of appropriate control to prevent intentional abuse of this newly discovered power. I feel it is essential that a governmental mandate authorize control, within this nation, to a council of superscientists of many disciplines within the genetic engineering spectrum. There must be provision for full disclosure to this group of the types of ongoing experimentation, as well as for unannounced peer sight surveys and inspection with the explicit intent of ensuring compliance. There must be a deterrent gradation of sanctions, quantified with a degree of safety breach, and applied to all parties concerned, including, but not necessarily limited to, the primary investigator, his associates and the involved institution.

This, then, concludes my observations concerning the proposed guidelines for Recombinant DNA Research. May I again thank you for the opportunity of participating in a meeting that mentally stimulated me far beyond any that I have been associated with in recent years.

Sincerely,


Joseph J. Dodds, M.D.
Member, Advisory Committee to the Director
National Institutes of Health

JJD/jc



DEPARTMENT OF MOLECULAR BIOLOGY
WENDELL M. STANLEY HALL
BERKELEY, CALIFORNIA 94720

March 9, 1976

Dr. Donald S. Fredrickson
Director, National Institutes
of Health
Bethesda, Md. 20014

Dear Dr. Fredrickson:

Thank you for your letter asking for comments on the proposed NIH guidelines on artificially recombinant DNA. Unfortunately, the letter and information arrived in Berkeley after the Feb. 9-10 meeting, but I hope that additional comments are still in order.

Generally, I think that the January, 1976 version of the guidelines is a reasonable compromise between those of us who are deeply concerned about the potential hazard of this technology and consequently are in favor of stringent regulation and those in favor of pressing on with important experiments with minimal restrictions. However, there are two points that still bother me a great deal. First, the drastic reduction of protection for DNA fragments that have been "rigorously characterized" and are "free of harmful genes" strikes me as highly dangerous (p. 28). I think one can reasonably demand three things for lowered containment: (1) the DNA has been rigorously defined (e.g. the hemoglobin gene); (2) the DNA can be justified as not dangerous; (3) the cloned DNA does not contain any unidentified DNA of size sufficient to code for a small protein (e.g. M.W. about 5000). The January guidelines cover (1) and (2), but not (3); I believe (3) is important and won't stop good experiments (e.g. cDNA from hemoglobin mRNA). Furthermore, I favor reducing the containment one step in those situations and not essentially abolishing it.

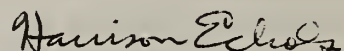
Second, I believe that the health surveillance aspect of this work is of critical importance and feel strongly that the January guidelines don't really deal completely with some critical issues. On p. 42, it says "Appropriate monitoring of personnel...should be conducted frequently." How about must for should (at least for P3 and/or EK2 situations)? In the next sentence, "evidence has suggested that ...nalR ara⁻, or mtl⁻ are useful..." Excellent in principle, but where is the implementing sentence that the bacteria (or better still, the vectors themselves) used must carry a detectable tag. To me, that requirement seems absolutely essential for an EK2 situation in which potentially hazardous genetic material will clearly be involved.

As a final point on a somewhat different topic, it seems to me that now may be an appropriate time to extend the safety concerns generated by the recombinant DNA work to other biological research, especially animal virus research. I continually hear horror stories of careless research in this area (as well as serious illness),

and I'm sure my experience is not unique. I would be tragic if the first human
tomor virus were identified through the death of some experimenters and their
families.

Good luck on the final stages of the guidelines. I hope it can be done soon.

Sincerely,

A handwritten signature in cursive script that reads "Harrison Echols".

Harrison Echols,
Professor of Molecular Biology

HE/sa

cc: DeWitt Stetten
Deputy Director
of Science

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LONDON, WC2A 3PX
Cables : Cancerch
Tel. : 01-242 0200

Dr. Paul Berg,
Dept. of Biochemistry,
Stanford University School of Medicine,
STANFORD, California 94305, USA.

10 March 1976

Dear Dr. Berg,

From the summary of the NIH committee guidelines for containing experiments with recombinant DNA reported in Science, it appears that there is no sub-classification of animal viruses with regard to the insertion of animal virus genes into E. coli. This is of concern to me since I plan to begin genetic and restriction endonuclease analyses on a group of insect viruses, the baculoviruses, which are potentially of great benefit to man and his environment.

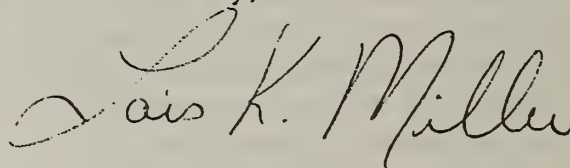
With the growing concern over the use of chemical insecticides for pest control, the baculoviruses are of great interest as biological pesticides. The Environmental Protection Agency has registered, after considerable safety evaluation tests, a baculovirus of the cotton bollworm (trade name Elcar). Baculoviruses specific for other pests are currently being developed for commercial production; the baculoviruses are species or occasionally genus "specific". Extensive safety testing on a variety of species (avian, piscian, mammalian, non-target invertebrates etc.) have been completed and in no instance were ill effects observed. The tests included potential for replication in mammalian cell cultures (including a variety of human lines), carcinogenicity and teratogenicity. The EPA has been cautious in registering these viruses and I know of no other viruses which have been so extensively studied with respect to safety.

Although I have no immediate plans to clone baculovirus genes in E. coli, recombinant DNA techniques are potentially useful for studies on the genetic organization of these viruses. The baculoviruses have a large DNA genome (90×10^6 daltons). The nucleocapsids are enveloped and embedded in a crystalline protein matrix which confers several unique properties allowing rapid and extensive purification of the viruses. There are over 300 reported baculoviruses infecting species within five insect orders. Within two years time, I hope to begin experiments aimed at recombining closely related viruses of pest insects. The purpose will be to obtain more effective pesticides with concern for safety.

Considering the tested safety of baculoviruses for man and his environment and the potential benefit of further research on these viruses, I would like to suggest the reduction of containment requirements from $P_4 + EK_2$ to $P_2 + EK_2$. The $P_2 + EK_2$ containment is cautious in comparison to the shotgun experiments with invertebrate DNA ($P_2 + EK_1$) which will surely involve introduction of viral genes harbored by the insects. I also wish to point out that agricultural research frequently does not have the funds to provide P_3 or P_4 containment facilities. For my part, I will be working in the Department of Bacteriology and Biochemistry, The College of Agriculture, The University of Idaho, Moscow, Idaho 83843 beginning July 1976. Finding funds for P_3 or P_4 containment will be difficult. Most of the research on these viruses are done at similar institutions.

Can you provide help or advice in regard to the reduction of containment requirements for baculovirus research? I am most willing to document this case and write innumerable letters to appropriate people if necessary. I appreciate your attention to this matter.

Sincerely,

A handwritten signature in cursive script that reads "Lois K. Miller". The signature is fluid and elegant, with the first name "Lois" and last name "Miller" clearly distinguishable.

Lois K. Miller

Bruce H. Goodman
21-15 Utopia Pkwy
Whitestone, N.Y. 11357

21-15 Utopia Pkwy.
Whitestone, N.Y. 11357
March 10, 1976

Dr. Donald S. Frederickson
c/o National Institute of Health
Washington, D.C. 20016

Dear Dr. Frederickson,

This letter concerns the recent controversy over the announcing of the government's new policy on the new implementation in genetic engineering.

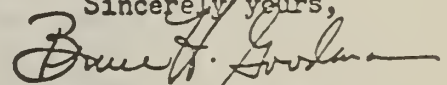
I think I may have a solution to the problem that will keep both the NIH and the people happy and safe.

One problem: The security of the laboratories is the question: whether the laboratory where the work would be done will be sufficiently secure against leakage and contamination.

1) First, as stated in the article by Judith Randal, of the News Washington Bureau (in the N.Y. Daily News, Feb. 26 & 27), the work would be done in the biological safety cabinets.

2) Then, to insure the containment of a possible leakage of this newly acquired bacteria into the environment, the laboratory should be equipped with a gamma irradiator. In other words, the laboratory itself will be one big gammator, closed off. Around the lab will be a large variety of highly sensitive detectors to detect leakage of any new microorganism. If the detector should register some leakage from these biological safety cabinets, an alarm would go off, and the whole staff, who are wearing specially coated outfits, go into a decontamination chamber. However, as soon as they have evacuated the lab, the lab is sealed tight, and the gamma radiators are turned on for a period of time. This will ensure the killing of the dangerous microorganisms.

Sincerely yours,



Bruce H. Goodman



THE UNIVERSITY OF TEXAS AT AUSTIN
LYNDON B. JOHNSON SCHOOL OF PUBLIC AFFAIRS
AUSTIN, TEXAS 78712

March 26, 1976

Dr. DeWitt Stetten, Jr.
Deputy Director for Science
Department of Health, Education,
and Welfare
Public Health Service
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Stetten:

I am constrained to write to you for two reasons:
(1) I cannot be present at the April 1-2 meeting; (2) I have concerns about safety in recombinant research that reach beyond issues to be discussed at the meeting. Moreover, you have requested that I send you a memo and that it treat particularly problems of implementation.

1. Technical Elements in the Guidelines

I left the meeting on February 9 and 10 appreciative of the high commendation for my colleagues on the Advisory Committee for the product they had produced and the consciousness of the public welfare that had prevailed in their deliberations. At the same time I say that the standards were in a number of instances not high or strict enough to win acceptance by informed professional persons, and by lay persons in addition to myself. I believe that the Advisory Committee should opt for strictness at this stage, first to win professional and lay acceptance, and second because experience in other programs shows that it is more difficult to tighten than to relax restrictions. Already, we have seen this problem raised with respect to use of cloned materials meeting Asimilar standards, but not those in the present guidelines.

a. The comments confirmed my impressions that there was little physical protection in P2 laboratories. Dr. Barkley's committee will undoubtedly add clarity to the definition of a P2 laboratory and perhaps also tighten the requirements for such a designation. Beyond this, the issues presented by

March 26, 1976

Dr. Frederickson for consideration at the April 1-2 meeting focus attention on the problem. My own vote, if I were present, would be in favor of raising the requirement to P3, or the higher level of EK2 in biological containment, in each of the instances where the issue is posed.

b. The meeting brought attention again to insects, embryonic DNA, SV40 DNA, certain hazards with prokaryotes, and whole plants. The suggestions in the issues posed by Dr. Frederickson for strengthening the guidelines on these matters appear to this lay observer to be desirable, reasonable, and adequate.

2. Implementation by NIH

Preparation of guidelines is only a first step; the big problem for the future is adherence to the guidelines. It would be very unfortunate if the committee's labors were unrewarding because of non-adherence, and also if such nonadherence led to imposition of controls that would frustrate scientific research. Adherence will be a major problem for a variety of reasons: ^{some persons will be careful} some will want to do recombinant research even when the conditions set by the guidelines cannot be met; and academicians are reluctant to "supervise" their colleagues. To make the guidelines effective on grantees these things appear to be necessary:

1) Broaden the local committee to include some one responsible for occupational safety, a student, a representative of the University administration, perhaps an informed person from outside the University.

2) Require certification on grant applications that conditions in the guidelines have been or will be met, such certification to be made by the investigator, the committee, and the University. The last of these is important because the University's reputation and its liability to suit will make its administrators careful.

3) Require recertification of compliance with guidelines at periodical intervals.

March 26, 1976

- 4) Reserve the right of NIH to make visitations.

I proposed in the December meeting of the Advisory Committee that NIH include in the grant document a statement that it reserved the right of visitation. I chose the word "visitation" because it can imply education and assistance, an implication not carried by the word "inspection." There is, however, some sanction in the possibility of visitation.

Dr. Frederickson's memo suggests consideration of NIH assessment of P4 facilities. There may be advantages in such assessment, but there may also be a question of NIH's legal authority. This leads to the next type of problem.

3. Implementation beyond NIH's Jurisdiction.

NIH's jurisdiction has served as a base for development of guidelines, but is inadequate alone for adoption and implementation of safeguards. Brief comments follow successively on policy needs and administrative arrangements.

- a. Several policy objectives can be identified:

- (1) Legislating the guidelines for all laboratories in which recombinant research is conducted.

For government research laboratories and grant agencies, this might be accomplished through agency adoption of the guidelines or possibly through a presidential order. Legislation would probably be required to make the guidelines binding in all private laboratories, and in instructional demonstrations in public and private schools.

- (2) Educating all biological scientists in research or instructional activity on the existence, importance and availability of the guidelines. Can this education, and the development of strong peer group attitudes toward compliance with the guidelines, be achieved through the scientific press alone? Or will the collaboration of government agencies in distribution of information be required? Must the education extend not only to scientists, but to occupational health and safety agencies and associations, and to public interest groups?

(3) Representing the United States in informational exchanges and negotiations with scientists or governments in other countries.

(4) Obtaining adherence to the guidelines in all recombinant research in any kind of institution or by any person in the U.S.

This problem cannot be evaded. The public and its representatives in government will probably believe that education and peer opinion alone are inadequate protection. The possibility of licensing of laboratories or researchers, or materials, has been suggested. Licensing is a severe requirement because it implies approval or nonapproval; and hence possibilities for delay, frustration of scientific initiative and progress, and bureaucratic limitation of freedom. There are other alternatives, less severe and less likely to impair freedom in research. Two deserve reference as possibilities:

One is a monetary penalty for violation of the guidelines. The other is a registration requirement, sanctioned by a monetary penalty. A registration requirement would have two advantages: it would provide knowledge in the science community of all recombinant research under way or to be undertaken; it would be an effective means of obtaining compliance with the guidelines.

The registration would not delay research, nor require approval, and could be made on a rather simple form. It would tell the nature of the project, the materials used and the laboratory type; it could also certify that all requirements in the guidelines were known and would be followed.

(b) It is not likely that the policy objectives can be achieved without additional administrative arrangements. Immediately, voluntary adoption of guidelines by other agencies for their grants or inhouse research, or requirement by executive order of use of the guidelines, may be achieved. But this would not reach research conducted without the aid of national funds. Moreover, the agencies are not likely to accept voluntarily guidelines, and amendments thereof, for a long period without their participation, or extension of NIH's authority.

DeWitt Stetten, Jr.

Page 5

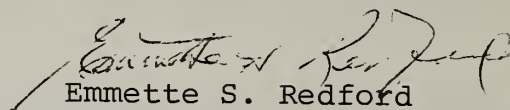
March 26, 1976

A legal jurisdiction with comprehensive authority--extending to all relevant parts of the government and to private research laboratories--will probably be established at some not very distant time. The appropriate officer to receive this jurisdiction is apparently the Secretary of HEW. HEW contains NIH, Food and Drug Administration, varied public health services and controls, and even means of reaching all educational institutions. No other department of the government concentrates so large a portion of the activities potentially related to recombinant DNA research. No other department is as well prepared by function and experience to balance the claims for freedom of research and advance in biological science with the claims for public protection. It could have a council with representation from other agencies, advisory scientific committees, and authority to delegate functions - including amendment of guidelines.

NIH has shown initiatives on recombinant DNA research that have merited commendation; its additional responsibility (probably already met) is to inform higher officials of the government of the broader problems not within its jurisdiction. These deserve consideration, with leadership from HEW, at the White House level.

These comments are sent without the benefit of discussions with other persons studying the problems. They are suggestive and perhaps contain something useful.

Sincerely,



Emmette S. Redford
Ashbel Smith Professor of
Government and Public Affairs



OFFICE OF THE PROVOST

CAMBRIDGE, MASSACHUSETTS 02139

March 29, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Public Health Service
Department of Health, Education, and Welfare
Bethesda, Maryland 20014

Dear Don:

Thank you for your kind and thoughtful letter of March 17. The meeting that you ran so convincingly--both as a scientist and a responsive policy maker at the highest level of public service--will always stay in my mind as a memorable event (even though I was sick during it).

Allow me to add two comments: (1) I regret that we did not have time to explore fairly fully the role of institutional committees--given the almost ideological differences that exist between and even within institutions, there will be a need for effective and continued interaction at a national level. (2) The NIH Recombinant DNA Committee will obviously continue to monitor the evolving situation. It may however be worthwhile to consider whether the Director's Advisory Committee should not play a role related to the broader, non-technical issues that were raised at Asilomar, and at the February meeting.

With warm personal regards,

Cordially,

Walter A. Rosenblith
Provost

March 29, 1976

Dr. W. Emmett Barkley, Director
Office of Research Safety
Office of the Director, NCI
Building 41, Room A107
National Institutes of Health
Bethesda, Maryland 20014

Dear Emmett:

You have asked whether I have any comments on the memorandum concerning recombinant DNA. This memorandum was written by the Boston group to the Director of NIH after they had reviewed my summary of "The Detrick Experience as a Guide to...P4..." (20 January 1976). The memorandum is clear and straightforward, but, like so much of the published reports in SCIENCE and elsewhere, it leaves me with an impression that the biological hazards have been hastily assumed, without critical examination by enough microbiologists experienced in the study of infectious disease.

In the field of infectious disease (omitting application of recombinant DNA research to insects and plants), I have been trying to imagine the successful use of recombinant DNA that would create a microbiological biohazard greater than any already known. I cannot do so. Possibly this is because I do not understand the full scope of the proved possibilities. Nevertheless, my view is (1) that each project should be conducted with precautions appropriate for the most dangerous biological reagent used in the experiment (rule of the most dangerous component, as in Attachment II of the 29 December 1975 NIH minutes), and (2) that an assumption of a resultant recombinant of even greater hazard would require a knowledgeable discriminating evaluation before it is accepted.

For instance, mention has been made of an E. coli-botulinum toxin hybrid. It seems to me it is most unlikely that it will be possible to cause an aerobic vegetative cell to produce *in vivo* a toxin formerly produced *in vitro* by an anaerobic spore former. Even if such a microorganism were formed, most likely the situation would be as with Cl. tetani, which long has been known to be present in the gut of the horse without production of toxigenic effect.

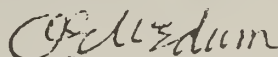
Dr. W. Emmett Barkley
Page 2

March 29, 1976

On this point, and in any other proposed recombination, there are experts (e.g., on Cl. botulinum) whose opinions should be sought. I assume this would be a responsibility of the proposed institutional biohazard committee. Development of antibiotic-resistant strains also has been mentioned. I fail to see any increased hazard in this sort of laboratory demonstration in view of the long history of non-transmission of laboratory-acquired infections to the general public. Furthermore, medical laboratories serving or associated with hospitals see antibiotic-resistant strains frequently. Any spread of them does not originate from laboratory personnel, but from the patient. To my knowledge, hospital laboratory personnel with laboratory-acquired infections have not been the cause of infection of persons outside the laboratory. One possible exception may be serum hepatitis.

Consequently, although development of an E. coli that will not replicate or survive in the human gut is a praiseworthy goal, I do not believe research on recombinant DNA molecules should be delayed until such a strain is available, when one considers the tremendous variability of the human gut among different persons and even within one person at different times.

Sincerely yours,



A. G. WEDUM, M.D.
Consultant, Project 4
Litton Bionetics, Inc., FCRC

AGW:eab

GIANT FOOD INC.

BOX 1804 · WASHINGTON, D.C. 20013

ESTHER PETERSON
VICE PRESIDENT
CONSUMER PROGRAMS

March 29, 1976

Dr. Donald S. Fredrickson
National Institutes of Health
Public Health Service
Department of H.E.W.
Bethesda, Maryland 20014

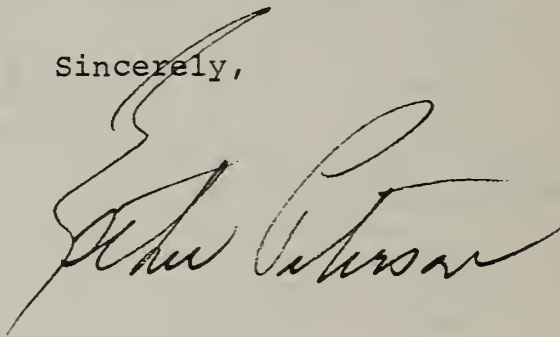
Dear Dr. Fredrickson:

Thank you for your letter relative to the Advisory Committee on the Recombinant DNA Guidelines. It was indeed a stimulating experience for me, and I appreciate being involved.

Should there be an occasion where I could be helpful in the future, I hope you will call on me. I am going over the reports with great interest. Good luck to you in the difficult decisions you must make.

Sincerely,

EP/sz

A handwritten signature in dark ink, appearing to read "Esther Peterson". The signature is fluid and cursive, with a large, sweeping initial "E" and a long, horizontal flourish at the end.



the University of Alabama in Birmingham / UNIVERSITY STATE OF ALABAMA
the Medical Center / DEPARTMENT OF MICROBIOLOGY

April 5, 1976

Dr. Donald Fredrickson, Director
National Institutes of Health
Bethesda, MD 2;0014

Dear Don,

Many have voiced concerns that institutions in the private sector may not adhere to the NIH Guidelines for Recombinant DNA Research and the application thereof to problems in medicine, agriculture, etc. At the recent NIH Recombinant DNA Molecule Program Advisory Committee meeting, I came up with a potential solution to this problem which I would like to call to your attention if this has not already been done; namely, pharmaceutical and industrial concerns are interested in maintenance of a good public image and are indeed interested in utilizing approved EK2 hosts and vectors. In this regard, I have already received numerous requests from commercial institutions for a stock of χ 1776. I thus propose that NIH assume responsibility for distributing these safer EK2 hosts and vectors and in filling requests, obtain a memorandum of understanding signed by an appropriate executive officer of the institution or by a responsible individual at an academic institution prior to distribution of the strains and vectors. In this manner, NIH could obtain compliance with its regulations even by those who are not provided with research funds by NIH.

Best regards.

Sincerely,

Roy Curtiss III

RCIII:kb

cc: Dr. DeWitt Stetten

[511]

STANFORD UNIVERSITY MEDICAL CENTER

STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG

*Jack, Lulu and Sam Willson
Professor of Biochemistry*

April 6, 1976

Dr. Donald S. Fredrickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Don,

I realize that you have labored long and hard to understand the issues and arguments that comprise the Recombinant DNA controversy. They are indeed complex and contentious, all the more so because of the paucity of data relevant to resolving them. But you have raised some interesting and important questions for the committee to consider. As I write this, I know that you have already met with the NIH Committee and received their comments; but for what they are worth to you, here are my views on some of the questions you posed:

A. Methods of Containment

a) I am puzzled by the difficulty several commentators had with the concept of physical containment, particularly because it can be defined more precisely than any of the forms of containment that have been discussed. In fact, it is the only tried, tested and accepted means available for containing dangerous pathogens; physical containment has been relied on for studies with hoof and mouth disease, Herpes B virus and the potential pathogens that might have been brought back from the lunar surface. I have learned that the British equivalent of the NIH Recombinant DNA Committee has opted to rely entirely on physical containment because experience with it has been so extensive and successful. Admittedly it is subject to human error but that only means that training and vigilance in the use of that containment facility must not be neglected. In any case I believe that Barkley's expanded explanation of physical containment should alleviate those concerns.

b) I agree with your suggestion that experiments to be carried out in P4 facilities merit special attention by individuals who understand the uses and limitations of such facilities. I can imagine such a panel of experts periodically visiting and examining the special facilities but what of the instance of a University's or Institute's own P4 facility? Who would supervise and monitor these once they have been certified as P4 laboratories?

B. Prohibited Experiments

a) I believe that a prohibition of all experiments which create organisms resistant to any antibiotic irrespective of its use in medicine or agriculture would have little merit. What danger would it head off? Why should one want to proscribe experiments involving antibiotics that are nearly useless for therapy but provide convenient selective agents for certain genetic manipulations? I believe that we should try to limit or prevent the creation of a) species of bacteria with antibiotic resistance properties not now known to occur or to arise by any natural recombination process (conjugation, phage mediated transduction or DNA-mediated transformation); and b) species of bacteria with combinations of antibiotic resistance determinants that are not known to occur or arise under the conditions mentioned above. With organisms of low or no pathogenicity P3 facilities seem adequate. But I would confine experiments in which single or combinations of drug resistance determinants are to be introduced into pathogenic bacteria to special P4 facilities or if the organisms were particularly virulent and dangerous, they should be deferred for the present.

Specific questions

1. I find the use of the words present or future use very confusing. How could one anticipate future use of an antibiotic? And who would decide if there is to be a possible use of an antibiotic in the future?

2. I omit comment on paragraph 2 because I think I would accept the opinion of people like Falkow, Clowes, Cohen and Novick. My limited knowledge, however, says that there are very few antibiotic resistance determinants that do not occur or arise naturally in E. coli when the appropriate selection is made for them. If EK2 E. coli are used, then there is a very reduced likelihood of such experiments causing difficulty. I suspect we should be more worried about putting drug resistance determinants into organisms other than E. coli inasmuch as there is presently no corresponding requirement for biological containment. Such experiments should be done only in very secure physical containment facilities, but perhaps only after the development of suitable biological safeguards.

3. I assume that what your question asked was whether experiments with DNA from an organism already (not potentially) infected with a Class 3, 4 and 5 pathogenic agent should be prohibited. I believe it should.

C. Biological Containment Criteria Using E. Coli K-12 Host-Vectors

3. I believe this version of the guidelines should state that the Committee has, until further notice, the responsibility to certify for use any and all EK2 or EK3 vector-host systems. May I urge, however, that the guidelines acknowledge that with the experience and knowledge gained from the proliferation of EK2 and eventually EK3 vector-host systems, such certification can be assumed by more widely based scientific review groups, i.e., study sections.

D. Classification of Experiments Using the E. Coli K-12 Containment Systems

1. Embryonic material must be handled aseptically to qualify for lower containment.

2. I suspect that your proposal to increase the containment requirements for cloning DNA of cold-blooded vertebrates generated considerable flack at the Committee meeting. You state that there is not available evidence or arguments that support the previously recommended levels but I wonder what arguments there are for increasing them? I believe that with sterile embryonic or germ line DNA from such organisms, the P2 + EK1 requirement is reasonable (one should not underestimate the containment capability of EK1). There is the mistaken impression that anything in an EK1 system is sure to become widely disseminated but the Committee report goes to some lengths to point out that that is not true.

3. The whole business about insect pathogens and the possibility of contaminating the insect is beyond my competence so I won't comment (I do include a copy of a letter I recently received that is interesting in this context). But I do think the guidelines should take care not to include all lower eukaryotes with insects. Common yeast, Dictyostelium, tetrahymena etc. are certainly not in the same category as organisms which might themselves be or harbor potential pathogens. If all experiments with lower eukaryotes were required to use EK2, that would immediately eliminate the very important and appropriate procedures developed by John Carbon for cloning pro- and lower eukaryote DNAs in E. coli plasmids.

5. I believe the concern you express here should pertain to prokaryote DNAs from organisms that are pathogenic or make a toxic product; otherwise, I fail to see why EK2 should be required in place of EK1. Your suggestion to require EK2 for experiments involving DNA from prokaryotes that do not exchange their DNA with E. coli concerns me. However, your suggestion does include the possibility that the higher containment requirement can be reduced if the recombinant genes are not

expressed or if they do not change the character of the E. coli host. But these assurances are probably unrealistic because each recombinant would have to be reviewed in detail. Consequently, the guidelines would have to specify what properties could or could not be altered to qualify for lower containment; alternatively, some group would be reviewing such data to pass on requests.

6. Your suggestion to require the Recombinant DNA Advisory Committee to examine the data and approve or disapprove lower containment for cloned segments from shotgun experiments also worries me. Because the Committee is not in constant session, it could be a very complex business to get an opinion or ruling. An investigator in the midst of such an experiment would very likely be held up by a cumbersome machinery that would frustrate the entire process.

I think the guidelines should try to state as clearly as possible what level of containment should be used to work with the recombinant organisms per se and to distinguish that, if appropriate, from the containment requirements for work with the purified plasmid or phage DNA from such an organism. It's plausible that work with potentially hazardous live organisms should be confined to the containment designated for that type of experiment but the purified DNA can be worked on and characterized using lower levels of containment. That would allow people to do the cloning in a P4 facility, grow up the culture under secure conditions, isolate its DNA and take it back to a lesser containment laboratory. Only if the segment of DNA being cloned is "beyond reasonable suspicion" should organisms harboring that segment be grown outside of the originally required facility. If that was spelled out in the guidelines, there would be no need for delaying reviews by Washington.

7. I believe that cloning cDNA from an RNA tumor virus should require as stringent a containment as any DNA tumor virus; after all, it is the form of the viral genetic information that would interact with mammalian genomes. But your statement implies a ban on such experiments. It was my impression that experiments of that type were not forbidden but did require the most secure containment levels - P4 + EK2 or EK3.

8. I would recommend (but how can you require) that mitochondria be isolated prior to extraction of its DNA for cloning, generally the way it's done to facilitate purification of that DNA. The levels you suggest for primate organelle DNA (embryonic and non-embryonic) are stringent but not entirely unreasonable. I assume that non-primate organelle DNA is in a lower containment level, which is also reasonable.

E. Experiments with Eukaryotic Host-Vectors

1. Any comments I make regarding the use of SV40 DNA as a cloning vector could be construed as self-serving; therefore, I'm wary about proceeding. But let me try. Work with SV40 virus, its DNA and the respective defective forms (those being generated naturally during infections or constructed enzymatically in vitro) are now done in P2 or P3 facilities depending upon the concerns of the investigator. (There is no NIH guidance or requirements on this matter.) Defective genomes cannot multiply without helpers, consequently, if they escape they can infect cells only if they are coinfecting with a complementing virus. I have given quite a bit of thought to making hybrids in which prokaryote DNA segments are introduced into the SV40 DNA molecule in place of specifically excised segments of the viral DNA. I have been unable to conjure up data or a substantive reason to support a ban on such experiments or even to require that they be done in P4 facilities. It's interesting to note that not a single person thought that introducing bacterial genes into SV40 and thence into animal cells was potentially hazardous when we made the first such hybrid three years ago. What has changed since then? Only the level of scrutiny. Do not misunderstand: I agree that using SV40 DNA as a vector to clone random segments of mammalian or primate DNA provokes considerable concern (such experiments do require P4 facilities according to the guidelines) but I do not feel it is outrageous to perform experiments with prokaryote DNA inserts under P3 conditions.

However, I can see where dealing with emotional concerns is important to get things moving and, therefore, I would support, without conviction, a requirement for P4 conditions to carry out such cloning experiments. It is likely, I believe, to force people to switch to polyoma DNA as the vector and that may be safer in the long run.

I'd like to illustrate, however, how research could change things. For example, certain modifications in experimental design could permit considerably safer experimentation with SV40; we have recently isolated the origin of SV40 DNA replication (this consists of 500-600 base pairs of the genome total of 5000 but this could very likely be reduced to about 100 base pairs) and planned to use that origin segment as a vector to clone "foreign" DNA segments. In this way only that portion of the SV40 DNA chromosome would be involved in the cloning. Depending upon the size and "packageability" of such DNA recombinants, it could provide the safest eukaryotic vector available. My point is that we cannot shut off the opportunity for innovation through experimentation.

Dr. Donald S. Fredrickson
Page Six

F. Implementation

I am quite concerned with what the guidelines say initially about implementation. Once a bureaucracy is established, it will be difficult to undo it. I hope and know you agree that we don't have to suffer the consequences of overkill.

There are several concerns: 1) The needed expertise to review and judge questions of experimental design, hazard and what to do about each does not exist at too many institutions and, for some time to come, is likely to be uneven in quality at different locations. Therefore, empowering local committees to approve anything more than compliance with clearly written guidelines is fraught with danger; 2) The NIH guidelines should be clear and forceful enough to discourage local option to impose stricter or more lenient containment requirements. That would be most destructive; 3) Investigators should be encouraged but not required to seek advice from any experts on the local Biohazard committee. The investigator should have the responsibility for experimental design and assessment of risk and level of containment (this is reviewed by study sections or in special cases by NIH committee) and the local committee should be apprised of these evaluations, and be responsible for monitoring the facilities, activities and keeping records of progress. Because of the unevenness I spoke of above, I can't see how local committees can "share responsibility" in evaluating risk and determining adequate containment.

It seems reasonable for the present to have investigators in the field obtain clearance from the committee concerning clones made earlier. This will not be a recurring function after the guidelines are promulgated.

The suggestion of cooperation with EMBO along the lines you mentioned at the top of page 10 seems excellent.

I also concur with the suggestion about describing containment methods used in a proscribed project when the work is published.

Is it within the NIH's purview to recommend committees for industry, agriculture and defense? I can't see any reason against such a recommendation.

I have written at much greater length than I intended but I hope these thoughts are helpful to you in resolving the issue and getting things underway.

Dr. Donald S. Fredrickson
Page Seven

If you want to discuss any of these or other points, I'm at your disposal.

With best regards,
Sincerely,

A handwritten signature in cursive script, appearing to read "Paul". The signature is written in dark ink and is positioned below the typed name "Paul".

PB:af
Enclosure

DEPARTMENT OF BIOLOGY

Kline Biology Tower

April 9, 1976

Dr. Ruth L. Kirschstein
National Institutes of Health
Behavioral Sciences
Laboratory of Psychology
Bldg. 29, Room 516
Bethesda, Maryland 20014

Dear Dr. Kirschstein:

The Mammalian Mutant Cell Lines Committee at its recent meeting of 5-6 April, 1976 discussed the desirability of establishing a central facility for the storage and distribution of mammalian DNA sequences. The Committee fully endorses the recommendation which is described in the following paragraphs.

Over the last few years the value of having a central facility for storing and distributing characterized human mutant cell cultures has become increasingly clear. This central facility has permitted the preservation of characterized cell samples for rare genetic disorders and has allowed them to be supplied to investigators throughout the nation. It is currently widely used.

The recently devised techniques of cloning DNA put within range the isolation of clones of DNA covering substantial parts of normal and mutant human mammalian genome.

We suggest that a valuable national asset would be provided if a facility comparable to the cell culture facility were set up for the storage, characterization and distribution of human mammalian DNA clones. Such a facility, if properly conceived, could serve a much wider function than a mere repository. It would act as a catalyst to future investigations by making characterized material available to other investigators, by offering specialized facilities for training scientists, and by providing a place for them to carry out special experiments. A brief list of the functions of such a facility includes:

1. Storage and preservation of cloned DNA
2. Characterization
3. Replication for distribution
4. Distribution
5. Opportunities for visiting experimenters
6. Training
7. Data compilation
8. Primary research

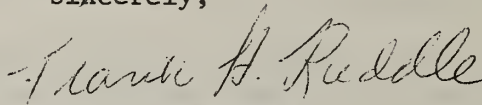
Dr. Ruth L. Kirschstein
NIH
Bethesda, Maryland
April 9, 1976

-2-

The value of such a facility depends on the imagination of its director and on the type of physical plant available. We suggest that NIGMS explore the possibility of encouraging the submission of proposals for the establishment of a national center for the storage, replication, and characterization of human and mammalian DNA clones. The center might take advantage of existing federally owned high risk containment facilities, or it might be an integral part of a facility planned by a university. The director of the facility ought to consider the opportunity the facility would provide for research as well as for its service function, and might want to consider an ambitious venture with several sections.

The opportunity for starting this type of DNA center at this early stage of a completely new episode in genetics seems to this committee to be too important to be missed. We would urge that some effort be directed towards exploring the utility and mechanics of such an endeavor and exploring other activities that might be logically associated with it. Obviously the deliberations and recommendations of the Recombinant DNA Advisory Committee will bear heavily on such considerations and the impact and advice of members of this committee would be extremely valuable. One would suggest, however, that any exploration of this suggestion include representation from this advisory committee as well as other organizations with overlapping interests (e.g. NCI and NIAID).

Sincerely,



FHR:mr

Frank H. Ruddle
Professor of Biology
and Human Genetics

Dictated by
but signed in his absence



State University of New York
99 Washington Avenue
Albany, New York 12210

Office of the Executive Vice Chancellor

April 14, 1976

Donald S. Fredrickson, M. D.
Director
National Institutes of Health
Department of Health, Education, and Welfare
Bethesda, Maryland 20014

Dear Don:

Many thanks for your letter of March 17 with respect to your difficult task of issuing guidelines with respect to Recombinant DNA research. I am sorry that it took me so long to respond.

I appreciated the opportunity to participate in the Advisory Committee meeting in Bethesda. I am much impressed by the fact that it was the scientific community that identified the potential public hazard of conducting research in the area of genetic engineering and that they, the scientists engaged in this research, were instrumental in calling a world-wide temporary halt to the research pending the development of guidelines which give attention to the public hazards involved and methods of safeguarding the researchers, their staff, and the general public. I am impressed with the thoroughness with which concerned groups addressed this problem and the thoughtfulness and thoroughness which you and your staff at the National Institutes of Health have addressed the problem. The research area itself is obviously beyond any competency which I might have to evaluate it and give you technical suggestions. You might find it useful, however, to have an objective outsider point up certain aspects of the proposed guidelines.

While the guidelines are very impressive, it strikes me that they constitute in large part the technical implementation procedures and elements of consideration for the implementation of a broad policy statement. However, no broad policy statement is made but has to be inferred. It strikes me that it would be appropriate to develop a carefully devised, short policy statement which would likely be appropriate for an extended period of time and then make the guidelines a technical implementation statement of that policy based on the present state of knowledge and subject to continuing revision as the state of knowledge improves.

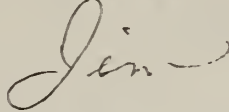
Much discussion occurred with respect to the enforcement arrangements for the guidelines. I would suggest that this is not the first order of business. It seems to me the development of a world-wide consensus stemming from the efforts and prestige of NIH dealing with the policy statement and dealing with the technical implementing guidelines should be the current objective; that a subsequent step would be the issuance of NIH regulations and regulations of other agencies that required the application of the policy and the implementing guidelines as a requirement for Federal funding. I am inclined to believe that if the scientific community gets behind the NIH approach, there will be a strong tendency for the enforcement to be voluntary and self-regulated. I say this because the scientists themselves engaged in this kind of research have identified the problem. Scientific recognition only occurs through publication in scientific journals. I seriously doubt that a scientist would seek publication of the results of his effort which were currently carried out in a manner not consistent with the guidelines. I seriously doubt that any scientist would engage in research in a manner that was knowingly not available for publication. Therefore, I do not think that the guidelines are essentially an enforcement document but rather are the thoughtful views of scientists as to how to protect themselves, their laboratory workers, and the public.

There is identified in the papers you distributed that high priority should be given to research in methodologies of containment and safety. I would not wish to belittle the importance of this research but I would not be inclined to place it in the highest priority. It strikes me that the highest priority should be accorded to research that endeavors to disclose the extent to which Recombinant DNA research is in fact dangerous and what that degree of danger is. I say this because I believe the essential difference between the problem of dealing with dangerous bacteria and dangerous infectious viruses is that those dangers are relatively well-known while work in connection with Recombinant DNA is conceived to have a potential serious danger to the health of humans, animals, and agriculture but the extent of that danger, if any, is unknown. From this reasoning I conclude that the basic policy statement would recognize that the scientific community undertakes careful safeguards in the conduct of research involving dangerous substances and that the degree of those safeguards are related to the known danger involved in exposure to the particular dangerous elements. Recombinant DNA is distinguished from the normal conduct of research in that it involves the creation of new substances which may be very dangerous to mankind and for ecology but the extent of that danger is speculative and unknown. Thus, the policy should be that where the danger is known, the normal process of safeguards related to the degree of danger should be applied. Where the danger is not known, but there is significant scientific speculation as to possible dangers, extraordinary safeguards should be employed until the degree of danger can be properly assessed.

April 14, 1976

I congratulate you on the excellence and thoughtfulness of the approach to this problem. I hope that these general comments may be of some assistance to you and I wish you success in developing a policy and implementing guidelines that provide adequate safeguards to the public and that generate an enthusiastic consensus of support among scientists world-wide.

Sincerely,

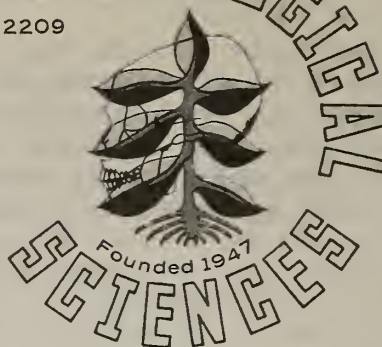
A handwritten signature in cursive script, appearing to read "Jim", followed by a horizontal flourish.

James F. Kelly

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES

1401 WILSON BOULEVARD • ARLINGTON, VIRGINIA 22209

15 April 1976



AIBS

703/527-6776

Dr. Donald S. Fredrickson
Director
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

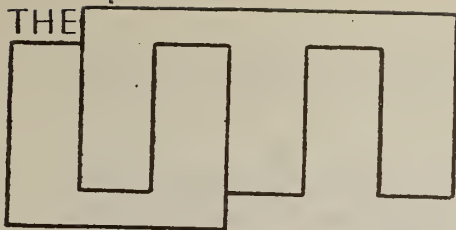
As we make our slow progress on the matter of DNA research, I wonder if the American Institute of Biological Sciences might not be of some assistance. There still are parameters to be considered along with the physical and biological controls or barriers. We should be thinking now of courses of action if something is allowed to get into the environment whether initiated through a disregard of guidelines or some oversight in other controls. What are the potential ecosystem impacts that pertain both to persons and other life forms? This takes us into a broader field than the traditional concerns. Maybe a study group or at least a symposium for an open discussion is indicated.

Secondly, where do we stand in the development of an International registry on all experiments? Every effort should be made to establish cooperation in reporting at an early date. It probably would be better to have a mechanism for approval prior to the undertaking of research to assure consideration of the greatest number of variables and options. The AIBS wishes to offer its services as you look ahead in the organization of mechanisms and procedures for the future of DNA research.

Sincerely yours,

Richard Trumbull
Executive Director

RT/ab



SCHOOL OF NATURAL RESOURCES

Samuel Trask Dana Bldg.
430 East University St.
Ann Arbor, Michigan 48104

UNIVERSITY OF MICHIGAN

April 16, 1976

Dr. Donald Fredrickson, Director
National Institutes of Health
U.S. Dept. of Health, Education & Welfare
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

I am writing to inquire as to whether you have prepared, or intend to prepare an "environmental impact statement" concerning your proposed issuance of guidelines for recombinant DNA research, and to request a copy of any such statement (or environmental assessment, or "marginal impact statement") when it is prepared.

It is my understanding that you have had an Advisory Committee working for some time developing guidelines for research which utilizes techniques for recombining DNA molecules into new forms of life, and that you will in the near future be preparing and issuing proposed regulations on this subject.

The National Environmental Policy Act of 1969 (42 U.S.C. 4321 et seq.) has established a framework of substantive policy goals that all agencies of the federal government are to pursue, supplementary to their other authorities and responsibilities. To insure that these goals are achieved, it requires, among other things, that to the fullest extent possible "the policies, regulations, and public laws of the United States shall be interpreted and administered in accordance with the policies set forth in this Act." More specifically, however, it also requires that before undertaking any "major federal action significantly affecting the quality of the human environment," the responsible federal official shall prepare and circulate to other agencies and to the public a "detailed statement," which describes the environmental impacts of the proposed action, alternatives to it, and other matters.

Applicable guidelines of the U.S. Council on Environmental Quality specifically list as "actions" within the meaning of the law "the making, modification, or establishment of regulations, rules, procedures, and policy;" and they express particular concern that such statements be prepared for actions "from which it is reasonable to anticipate a

April 16, 1976

cumulatively significant impact on the environment," and "actions, the environmental impact of which is likely to be highly controversial" (38 Federal Register 20550, August 1, 1973, Section 1500.5). Similar and more detailed guidelines have been established by the Department of Health, Education, and Welfare (General Administration Manual, Chapters 30-10, 30-11, 30-12, 30-15).

The issuance of guidelines or regulations for recombinant DNA research appears to fall clearly within both of the categories quoted above, and thus within the requirement that a detailed statement be prepared and circulated prior to their adoption. The direct impacts of such regulations would include at least the risk of inadvertent release of new and potentially dangerous forms of life into the environment; the indirect and cumulative effects could include a wide range of significant ecological changes, particularly if the dreams of some advocates of this research are realized in widespread industrial applications.

However, I am not aware of any statements of your intention to prepare such a statement, nor of any efforts yet made to do so, despite language in the CEQ guidelines that these activities should be undertaken "as early as possible and in all cases prior to agency decision concerning recommendations or favorable reports on proposals" (38 Fed. Reg. 20550, Section 1500.2).

I should appreciate, therefore, the following information:

(1) Notification as to whether any Environmental Assessment, Marginal Impact Statement, or Environmental Impact Statement has been prepared for this proposed action, or as to whether you intend to prepare such a document (and if so, which); and

(2) A copy of any Environmental Assessment, Marginal Impact Statement, or Environmental Impact Statement when it is prepared, for review and an opportunity to comment on it.

I would hope that such a document would give particular attention not only to the various impacts that might result, both locally around a research facility and generally as a consequence of pursuing research in these areas, and to the major areas of continuing uncertainty that exist; but also to careful discussion of major alternatives to these proposed guidelines. These alternatives include at least the alternative of (a) banning certain forms of this research; (b) delaying the pursuit of them until further research and testing of safety measures can be carried out; (c) limiting research to a few strictly controlled facilities; and (d) establishing broader guidelines or legislation to control such research by industry, the military, and other potential

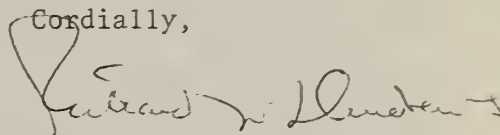
Dr. D. Fredrickson, Director

April 16, 1976

investigators rather than merely by those using NIH funds. In the latter regard, note that judicial precedents and CEQ guidelines do require discussion of "all reasonable alternative actions, particularly those that might . . . avoid . . . adverse environmental effects . . . including, where relevant, those not within the existing authority of the responsible agency" (38 Fed. Reg. 20550, Section 1500.8).

I appreciate your attention to this matter.

Cordially,

A handwritten signature in dark ink, appearing to read "Richard N. L. Andrews", written over a circular stamp.

Richard N. L. Andrews
Associate Professor

RNLA:jg

cc: The Secretary of Health, Education, and Welfare
The Assistant Secretary for Health
HEW Environmental Affairs Office
U.S. Council on Environmental Quality

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

School of Medicine
Department of Microbiology, SC-42
and Immunology

April 16, 1976

Dr. DeWitt Stetten
Deputy Director for Science
Department of Health, Education,
and Welfare
Public Health Service
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Stetten:

I wanted to put in writing one point of concern about the proposed guidelines for research involving recombinant DNA molecules that came out of the discussions at the meeting to which I was invited as a consultant. This concern relates exclusively to administrative aspects on pages 41 to 44 of the draft of the tentative guidelines. I consider the rest of the document to be superb and to give a very completely thought through series of recommendations which will, I believe, suffice to protect the public and the environment from even extraordinarily improbable events as long as they are followed - and thereby hangs my concern.

Experience of laboratory infections with pathogenic microorganisms shows that recognized precautions often have not been followed or have become eroded with time because difficulties have not previously arisen. I predict that if one visits clinical chemistry or serology laboratories in many large centers one would find a considerable proportion which are not using the most elementary recognized precautions for avoiding the known risk of hepatitis. On the other hand, where state regulations exist concerning the handling of Mycobacterium tuberculosis, and the laboratories are subject to inspection, the level of care used tends to approximate that which is recommended and which is needed for personnel protection. I use these examples to point up that recommendations without local responsibility for enforcement other than the judgment of the individual principle investigator are often relatively ineffective. This is likely to be compounded when precautions are invoked for theoretical hazards which have never been realized in practice.

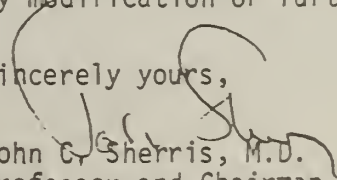
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I consider that the staff proposals for a replacement of section four were basically sound (although they could be simplified here and there), and disagree with the Committee's view that the local institutional role should be limited to advice and certification that appropriate facilities are available. Local involvement and over-seeing seems to me to be as essential in regard to recombinant DNA work as it is in the control of fire, chemical or radiological hazards or in the case of human experimentation. I believe that there must be continuing onsite appraisal by individuals other than the principal investigator not only of the suitability of facilities and training of personnel conducting the experiments but, in particular, to insure that the precautions that apply to a particular experiment are actually in use. The institution as the employer or sponsor of the investigator has, I believe, a legal and moral responsibility to concern itself with the nature and control of any potentially hazardous activity, and this is no exception. Actually, we are obligated in this state to play an active role under the requirements of the Washington Industrial Safety and Health Act, let alone under the requirements of OSHA.

I have had some experience with another situation which involves the control of real or potential dangers and the need for the application of strict rules and standards of containment. This is the question of the control of hospital infections. All the experience gained over the last couple of decades or so indicates that to expect the problem to be controlled simply by requiring the application of particular isolation and other techniques by physicians and nurses is unrealistic and doesn't work. There are endless opportunities for differences in judgment and rationalizations, and it has been found essential for an institution to have an active infections committee and an epidemiologist with considerable authority if procedures are to be maintained and nosocomial infections kept down to acceptable limits. I believe there are parallels here with what is likely to be found in the application of the guidelines.

I realize that there are many who would regard these views as applying excessive infringements on academic freedom and opening the door to local uninformed bureaucratic interference with the initiation and performance of important experiments. Should these views prevail, I urge that an investigation be undertaken to determine the degree of operational conformity to the guidelines as experiments begin to be undertaken around the country on a large scale. This should involve spot checks without advance notice by appropriately qualified NIH staff or advisors so that the level of conformity to the guidelines can be determined and data derived for any modification or further action that may be needed in the future.

Sincerely yours,


John C. Sherris, M.D.
Professor and Chairman

JCS/smb

April 21, 1976

Dr. Donald S. Fredrickson, Director
National Institutes of Health
Building 1, Room 124
Bethesda, Maryland 20014

Dear Dr. Fredrickson,

First, let me complement you on the whole way you have handled the Recombinant DNA guidelines. Your approach has been thorough and responsible, really giving a sense of listening to a variety of views. I can see that the delays to date, while undesirable, have been unavoidable.

There is one major issue to which I feel we as a committee have not addressed ourselves adequately, which was brought up by several of your correspondents. This is the question of proliferation of sites where such research is done, particularly of P3 and P4 facilities. Since fall, it has been apparent that this is a problem, and Emmett Barkley, Wally Rowe and I have been discussing the possibility of having a few national P3 and P4 facilities openly available. Most non-committee scientists to whom I have broached the idea like it, from Joshua Lederberg to scientists in many major universities to Rich Goldstein. This appears to be more an administrative policy decision than one which our committee would need to incorporate into guidelines.

I see many advantages of working in regional centers; at least for P3 and P4 experiments. For example: (a) It would be less expensive to construct and staff a few such regional centers than a proliferation of local ones. (b) The primary responsibility of the staff would be conducting potentially hazardous experiments, and they could be trained and tested much more carefully on the techniques than could a graduate student or technician for whom the actual cloning is only one small, rather technical part of a long-range problem. (c) The technology would then be much more uniformly accessible to qualified investigators from a variety of institutions, large and small, tending less to artificially create an "elite" - a major concern of Dr. Lederberg's. (d) It would be far easier to make sure that such facilities were not in vermin-infested buildings; in buildings dealing with patient care; near heavily-traveled corridors; etc. (e) Each organism of major interest could be "shotgun" - cloned one or a few times, with banks of clones maintained to be selected out by individual investigators, rather than having each "shotgun" experiment repeated hundreds of times. (f) The sites could be chosen slightly away from population centers, as at Ft. Detrick, to minimize the possibility of damaging law suits from people who think nearby recombinant DNA might be the cause of their ailments. (g) People selecting their clones in such facilities could be properly trained in the process in safe techniques for dealing with their purified clones at home. (h) It would be far easier then to be sure that one still had the proper

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Mar 23 1977

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Dr. Donald S. Fredrickson

Page 2

April 21, 1976

EK2 or EK3 strain uncontaminated.

Clearly, a policy where regional centers were funded in preference over encouraging a proliferation of local facilities would have its complications: decisions on priorities, necessity for long-range planning, wide-spread input and cooperation, travel expenses, a slight decrease in individual autonomy. I know some members of the committee feel that this would be an unwarranted barrier to research; for some institutions, "personal" labs probably are the answer. However, in general I think the advantages of regional labs far outweigh the disadvantages, and most of those with whom I have spoken outside of the committee seem to agree. I think the possibility at least deserves further exploration.

Sincerely,

Elizabeth Kutter

Dr. Elizabeth Kutter

Member of the Faculty, Biophysics

THE UNIVERSITY OF MICHIGAN

ANN ARBOR, MICHIGAN 48104

DEPARTMENT OF HUMANITIES

COLLEGE OF ENGINEERING

(313) 764-1420

21 April 1976

Dr. Donald Fredrickson
Director
National Institutes of Health
Bethesda, MD 20014

Dear Dr. Fredrickson:

We write to express our concern over several aspects of recombinant DNA research which we feel are of critical importance and to recommend that they be addressed by the Recombinant DNA Molecule Program Advisory Committee before the guidelines are disseminated to the NIH grantees and contractors.

In the first place, we feel that the data presented by Dr. Curtiss to the committee in April in his paper, "Construction, Properties and Testing of X 1776" raises serious doubts about the effectiveness of biological containment. Dr. Curtiss himself points out in the introduction (p.3) that this strain does not satisfy the criteria for a disarmed strain set down in the guidelines drafted at La Jolla. Specifically, the degradation of cloned DNA fragments is not guaranteed, and in fact, has not been tested by Dr. Curtiss since his experiments have been carried out on a host-vector system not containing cloned DNA. Furthermore, it appears from much of the data presented that the strain X 1776 can survive both in the dry state (pp.131-132) and in a variety of environments including tap water (pp.127-128) for considerable periods. In the light of this evidence, we think it is essential that the committee's discussion of the properties of X 1776 and its decision to certify or not to certify this strain as a disarmed strain be carried out in public. If it is not contrary to the letter of the regulations governing the conduct of the committee, it is certainly contrary to their spirit that this important issue be decided by mail, as was proposed at the April 1-2 meeting.

Second, the committee should write an Environmental Impact Statement as required by the National Environmental Policies Act of 1969. Dr. Richard Andrews of the University of Michigan School of Natural Resources has written to you about the applicability of this law.

Third, the committee should write a statement on the feasibility and costs of monitoring, both of laboratory personnel and of the environment. If effective monitoring turns out to be difficult or impossible, or beyond the financial means of most universities, then we think that you and the committee should

explore the implications of a situation in which legal liability will be difficult to establish. In particular, you should address the question of whether it is justified to initiate research before an effective monitoring system has been articulated in relation to the legal system. Our view is that the absence of such articulation violates the right of the public to security and access to proof of liability in the case of an accident.

Finally, we wish to emphasize the confusion and uncertainty which surrounds crucial aspects of DNA recombinant experimentation. Little is known about the nature and level of risk; the theoretical basis of the guidelines is questionable, as Robert Sinsheimer has pointed out; the efficacy of biological containment is not assured. In the light of these conditions, we think that proliferation of laboratories presents a grave risk to the security and well-being of the public. We recommend that you explore alternative policies for the research which would provide a much greater degree of protection, specifically, the possibility of limiting research to a few strictly controlled national facilities until the risks are better defined. As you are no doubt aware, scientists at a number of leading institutions have grave doubts about the advisability of proliferation. We think that there would be considerable support for mutual exploration of this alternative policy.

We would appreciate your attention to this letter and a response before action is taken to disseminate the guidelines.

Sincerely,

Arthur Schwartz
Professor of Mathematics

Susan Wright
Assistant Professor of
Humanities

Marc Ross
Professor of Physics
Director, Residential College

Robert P. Weeks
Professor of Humanities

Max Heirich
Associate Professor of
Sociology

(Signatures attached)

[Please address correspondence to the first two named]

Donald H. Michael

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School of Natural Resources
Dept of Psychology

Arthur Schwarz

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April 30, 1976

Dr. Donald S. Frederickson
Office of the Director
N.I.H.
9000 Rockville Pike
Bethesda, Maryland 20014

Dear Dr. Frederickson:

Several of us at the University of Wisconsin-Madison are working in the field of DNA cloning. Two matters in the latest version of the proposed guidelines (available to us in April, 1976) are of concern to us since they appear to depart significantly from the proposed guidelines (January, 1976) emanating from the Torrey Pines meeting.

1) We understand that the wording of parts of the guidelines proposed in January as they relate to the Biological Containment Criteria Using E. coli K12 Host-vectors may be changed as indicated here:-

* Pg. 18. "EK2 host-vectors - these are host-vector systems that have been genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. The genetic modifications of the E. coli K-12 host and/or the plasmid or phage vector should not permit survival of ~~the cloned~~ *a genetic marker carried on the vector, preferably on the inserted* DNA fragment in other than specially designed and carefully regulated laboratory environments at a frequency greater than 10^{-8} . This absolute measure of biological containment has been selected because it is a realistic measurably entity. Indeed, by testing the contributions of pre-existing and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible to substantially indicate, if not prove, that the specially designed host-vector system can provide a margin of biological containment in excess of that required.

For EK2 plasmid vectors, no more than one in 10^8 host cells ~~containing a chimeric plasmid~~ should be able to perpetuate the *vector and/or a cloned* DNA fragment under nonpermissive conditions designed to represent the natural environment either by survival of

*
~~Old-version~~ new version

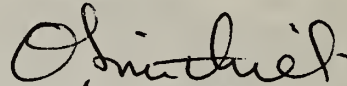
the original host or as a consequence of transmission of the *vector and/or cloned DNA fragment* by transformation, transduction or conjugation to a host with properties common to those in the natural environment."

Pg. 21. "For EK2 phage vectors, no more than one in 10^8 ~~recombinant phage particles~~ should be able to perpetuate *itself and/or a the cloned DNA fragment* under non-permissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring *itself and/or a the cloned DNA fragment* to a host (or its resident lambdoid prophage) with properties common to those in the natural environment."

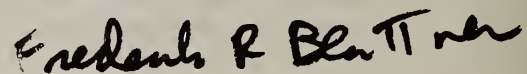
The new wording differs very significantly from the older form by its de-emphasis of measurements related to the propagation of the cloned DNA yet these are the only really meaningful measurements. The wording of the older form seems to us better, but whatever wording is ultimately used it ought to stress that the cloned segment is the element of concern.

2) We urge that before any proposed host/vector system is accepted as safely meeting the guidelines, for example for EK2, the proposers of the system should make their primary data available for public scrutiny, either by publication in a journal or by distribution in the NARSM circular. In this way, reviewing bodies who certify safer vectors would know that the relevant data had been sufficiently available for public criticism before certification was completed.

Sincerely,



Oliver Smithies
Leon J. Cole Professor of Genetics
and Medical Genetics



Frederick R. Blattner
Assistant Professor of Genetics



Harvey E. Faber
Post-Doctoral Fellow

OS:fm

xc: P. Leder; W. J. Gartland

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PASADENA, CALIFORNIA 91125

DIVISION OF BIOLOGY

May 3, 1976

Dr. Donald Fredrickson
National Institutes of Health
Bethesda, Maryland 20014

Dear Don:

I expect you have a few other matters to occupy your time in addition to the recombinant DNA issue. However, this is and will doubtless continue to be an important issue and I therefore feel obliged to comment upon Bernie Davis' recent letter to you on this subject (of which he sent me a copy).

Bernie takes issue with my "speculative suggestion" of a possible genetic barrier between prokaryotes and eukaryotes which may be of some importance in the prevention of genetic or viral interaction between these great classes of living organisms - and, if so, might be breeched only at considerable peril. His characterization of this hypothesis as a "speculative suggestion" is quite correct; the distinctions, as yet poorly defined, in the control of DNA replication and transcription between these classes may be of minor biological importance (indeed I feel obliged to hope this will prove to be the case). But I do not believe anyone can state that conclusion with confidence at this time. We just do not know. And I do not find the arguments in Bernie's letter particularly persuasive.

The possibility that some genetic exchange between prokaryotes and eukaryotes does occur at least at historical rates was previously proposed by David Baltimore. Obviously this possibility cannot be excluded but neither can the rate be defined. I do not wish to enter into a numbers game of 10^{-x} , although I could take exception to some of the x's used in Dr. Davis' letter. I would only point out that the (very small) likelihood that *E. coli* can take up and incorporate exogenous DNA is known to be grossly influenced by a) the culture conditions in which the cells are grown, b) the ionic conditions of exposure to the DNA, c) the condition of the surface components of the cell, d) the presence or absence of external nucleases, e) mutations affecting the cell surface, cellular nucleases, enzymes involved in recombinatory processes, etc. In these circumstances it is simply impossible to predict the likelihood of such events in the intestines of man or other animals. As Dr. Davis says, it is also impractical even to attempt to obtain an empirical estimate.

Dr. Davis also advances the evolutionary argument that we need not be concerned about possible hazards from organisms containing recombinant DNA since (in his view) opportunities for the formation of such organisms have occurred (repeatedly?)

Dr. Donald Fredrickson
May 3, 1976
Page 2

in the past and had such organisms had an evolutionary advantage they would be with us today. I really cannot accept this argument which - it seems to me - amounts to saying that everything that could have happened, in an evolutionary sense - has happened. I don't believe it. I do not believe there has been enough time nor opportunity to sample more than a small fraction of evolutionary possibility.

The fact is we have no predictive theory of evolution. We have only the beginning rudiments of a quantitative retrospective theory of evolution. As Dr. Davis knows well there are major scientific controversies over the relative importance of "neutral" vs. "advantageous" mutations, over the relative significance of mutations affecting structural genes as compared to mutations affecting regulatory genes, over the possible role of viruses as vectors for gene transfer, etc. etc. Current theories of evolution can hardly begin to cope with questions as to the importance of non-linearities, of coincident mutational events, of "critical mass" effects, etc. (e.g. One *E. coli* bearing a gene for botulinus toxin might in fact be at a disadvantage and perish. 10^7 *E. coli* bearing the gene for botulinus toxin might so change the ecology of an intestine as to be at an advantage).

We know that homo sapiens evolved during the last 10^7 years. I do not believe anyone could tell you why homo sapiens did not appear 10^8 years earlier - or why his appearance was not delayed another 10^8 years. There is no reason to believe that everything that could have happened in evolution has happened. (Indeed many of the proposed benefits of genetic engineering are based specifically on that presumption).

I dislike taking an obscurantist role. But I believe that on a matter of considerable public concern such as this it is as important to make clear our ignorance as to make clear our brilliance. There is a lot we do not know and we should acknowledge such. Having done so, an administrative decision can - and of course must - still be made.

With best wishes,

Bob

Robert L. Sinsheimer
Chairman

cc: Dr. Davis
Dr. Stetten



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May 5, 1976

Dr. Donald Fredrickson
Director
National Institutes of Health
Bethesda, MD 20014

Dear Dr. Fredrickson:

I have been following very closely the development of recombinant DNA research guidelines by the Recombinant DNA Molecule Program Advisory Committee.

At the present time, I believe the committee has failed to fulfill its proper function by neglecting to file an environmental impact statement in accordance with the National Environmental Policies Act of 1969. The filing of the impact statement will allow a greater number of interested groups and individual citizens to comment on the problems inherent in recombinant DNA research.

Another issue that needs to be addressed is the problem of determining legal liability for accidents resulting from recombinant DNA research. A system to monitor the research has to be developed so as to guard against accidents and to insure that the research universities and grantor agencies are taking the necessary safety precautions. Only through careful monitoring can fault be determined if an accident does occur.

I trust that the committee will continue to explore methods of insuring safe experimentation and that alternative experimental procedures to those presently used will be recommended until biological containment is feasible.

Yours truly,

Eric Cohen
Research Director

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : Director, NIAID
Through: Acting Deputy Director, NIAID
Acting Scientific Director, NIAID *SMC*

DATE: May 5, 1976

FROM : Chief, Laboratory of Viral Diseases, NIAID

SUBJECT: NIAID Risk Evaluation Program on Recombinant DNA Molecules

As you know, the NIH guidelines for research on recombinant DNA molecules will be issued in the near future. In large part, the great difficulties faced by the NIH Recombinant DNA Program Advisory Committee in devising these guidelines was due to the absence of relevant data on what the risks might be. The guidelines are considered by many, including me, to represent a very conservative, stop-gap mechanism which is temporary, pending the acquisition of data which will either allow relaxation of containment or will substantiate the existence of a real biohazard. In my opinion, the success of the guidelines will depend initially on the good will and sense of fairness of the scientists interested in doing such research, and subsequently (after a year or two) on their own evaluation of whether the restrictions are really indicated. I strongly suspect that within two years after issuance of the guidelines, if there is no concrete evidence that a biohazard can develop, violations will be so rampant that heavy handed external regulation will be imposed. The problems that this would entail, plus the polarizations that would develop between different groups of scientists, and between scientists and legislators, would produce disastrous consequences for NIH..

Accordingly, I see it as a matter of much urgency that NIH assume the responsibility of ensuring that the evaluation of possible risks be done in an expeditious, competent, and visible manner.

To date, the collection of data on risk has been fragmented and rather haphazard. Some of the laboratories working in bacterial systems have looked at colonization of people by K-12, the occurrence of λ -sensitive E. coli in man, and, of course, the ability of E. coli to translate eukaryotic DNA; however, these are not part of any coordinated program. The polyoma-E. coli experiment of Malcolm Martin and me also represents a significant step in this direction, but it is only a small part of the needed research. One relevant initiative of the Program Advisory Committee was the appointment, in December 1975, of a subcommittee consisting of Drs. Szybalski (Chairman), Hogness, Spizizen, and me, to deal with risk evaluation. Our subcommittee has not been able to examine the problem, and I do not think that a widely dispersed subcommittee could be expected to

supply the focus and coordination, or to establish the administrative arrangements that will be needed to get the research under way. Rather, I envision its function as being to advise on and support initiatives taken elsewhere.

I think it would be very much in the interest of the NIH, and of science in general, for NIAID to assume the responsibility for a program on risk assessment. I do not have a clear picture of what this would entail; certainly competence in microbial genetics and bacterial pathogenesis, possibly animal virology, and facilities for P₄ level research would be needed. An intramural operation, possibly located at Fort Detrick, should be considered.

This problem offers a crucial opportunity for NIAID to become more strongly identified with recombinant DNA research, to supply urgently needed leadership, and to perform an essential and urgent function. I think there would be immense support for such a program, from all levels of interested people.

A copy of a letter outlining some of my thoughts on the scientific aspects of risk evaluation is enclosed.

A handwritten signature in dark ink, reading "Wallace P. Rowe". The signature is fluid and cursive, with the first name "Wallace" being more prominent and the last name "Rowe" following in a similar style.

Wallace P. Rowe, M.D.

1 Encl
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FRIENDS OF THE EARTH 72 JANE STREET • NEW YORK, NEW YORK 10014 • (212) 675-5911

May 17, 1976

Dr. Donald Fredrickson, Director,
National Institutes of Health
U.S. Dept. of Health, Education & Welfare
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

We understand that you are considering adoption of the NIH Advisory Committee proposed guidelines for research involving the recombination of DNA molecules, preparatory to drawing up and issuing regulations, and would like to make some comments and suggestions at this time regarding the guidelines and regulations.

Friends of the Earth is a national environmental organization, with many foreign affiliates, and has been active at all levels of government in the key environment, conservation and natural resources issues of our day, including many which have direct bearing on human health and welfare such as nuclear power. Although at this time we are not qualified to scrutinize the specifics regarding DNA guidelines and containment (others have of course done so), we do have deep concern for the as yet unknown risks, the unquantifiable risks, and, not least, the broader social, ethical, political and evolutionary implications of genetic engineering in general.

We have been particularly struck, in the small, preliminary steps being taken to deal with genetic engineering problems, with the parallels to the nuclear power controversy, which of course received no public debate or scrutiny for the first twenty years of its commercial existence. Both nuclear power and genetic engineering seem to be proceeding on the assumption that they must proceed, yet no public debate has been initiated on genetic engineering even now as the impetus grows. We believe that all debate on this subject should start with the basic question: should genetic engineering (or any branch of it) take place at all?

Further, since genetic research and its human or non-human applications are without a doubt major actions, particularly because HEW guidelines are involved, we believe that NIH should prepare an environmental impact statement in compliance with the National Environmental Policy Act. However, we would hope that this impact statement would not be limited only to the biohazards associated with recombinant DNA research but with the broader spectrum of issues as raised above. In this regard we would like to point out to you some of these areas of concern to which any proper EIS should be addressed.

1) Evolutionary

What are the consequences of interfering with or re-directing evolution and natural selection? Of reducing diversity in the gene pool, as in gene therapy? Should recombinant DNA proceed without our knowing whether genetic barriers between species are adaptive or accidental, positive or neutral? Shouldn't population geneticists such as Ernest Mayr be consulted on this?



2) Legal liability

In the event of viral infections or contagions affecting the public, who would be liable: the experimenter, the laboratory, the university? Would existing malpractice insurance suffice? Would an unlimited liability law be advisable or necessary to protect the public?

3) Regulation, inspection and enforcement

Proposed NIH guidelines lean on self-regulation, and seem to be set up to promote and permit unhampered research, not to question or impede it if and where necessary. Both self-regulation and government regulation have inherent problems in that the real hazards might be underplayed or are assumed to have solutions (with problem and solution defined by scientists, not the public). Status and prestige of important biological research carry an impetus that might tend to denigrate public criticism or concern, and could lead in turn to the rationalization that scientific freedom and research are being "stifled". Scientists need reminding that the benefits of their research are worth no more than the public thinks they are worth, and that public interest research carries heavy responsibilities that may require restrictions and accountability beyond that which scientists believe adequate.

4) Social and ethical problems

The first question that needs to be asked - and answered - regarding cost vs. benefit comparison is: who benefits? who is at risk? And if these can be defined, who decides that the risk is justified? Who decides which genetic traits are desirable or undesirable? Who decides when these traits or medical problems become serious burdens on the community or society, justifying complex costly and possibly dangerous solutions? What are the dangers - economic and scientific and social - of overlooking or excluding simpler solutions to social problems (population control vs. better nitrogen fixation; genetic counselling or enzyme therapy vs. gene therapy, etc.) Is there a danger where complex technologies are involved that the experts may tend to dominate out of vested interest in promoting such "beneficial" technologies? In brief, is genetic manipulation the proper solution to problems that have both a social and medical basis?

5) Political

It has been said that modern technology is replacing the biosphere with the technosphere, abandoning reliance on naturally evolved, internal controls in favor of external technological ones. What are the implications of this kind of national policy? Furthermore, common interests in scientific research may make scientists hesitate to "blow the whistle" on colleagues or do anything that might threaten research grants. Recipients of such grants may unconsciously toe the line even without external coercion or threat of reprisal. What are the implications of increasing centralized control over information, technology application, especially with regard to national social goals?

(more)



6) Benefits

Despite the clarion calls heralding scientific miracles, the benefits of genetic engineering remain to be demonstrated in society at large. In vitro experiments, like computer models, are one-dimensional as far as humans (and human society) is concerned. Are the promised benefits merely pie in the sky or capable of fulfillment? Do they fill demonstrated health needs or only those needs defined by the scientists involved? Are the so-called benefits possibly dreamed up merely to justify research? (One can always find benefits for nearly everything). And if scientists really believe their work will benefit society in important ways, indicating their sense of social responsibility, why should they object to scrutiny and controls as adjuncts to their sense of responsibility?

We hope that you will give serious consideration to the broad spectrum of social, ethical and scientific issues raised by genetic research before any irreversible commitments are made to apply this research to human beings and society, and we would appreciate receiving a copy of your environmental impact statement when it is completed so we may comment on it.

Sincerely,

Lorna Salzman

Lorna Salzman
Mid-Atlantic Representative,
Friends of the Earth

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Jeremy J. Stone
Director

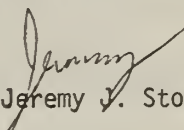
May 18, 1976

Dr. Donald Fredrickson
Director, NIH
Building 1, Room 124
Bethesda, MD 20015

Dear ^{Don} Dr. Fredrickson:

Remembering your interest in our April publication on DNA recombinants, and your heavy responsibilities in promulgating the guidelines, I thought you might welcome an advance copy of our straw poll results drawn from those of our 7,000 members who were interested enough to respond.

Sincerely,


Jeremy J. Stone

JJS:jes

[545]

National Council Members (Elected)

Ruth S. Adams
David Baltimore
Nina Byers
Rose E. Frisch
Arthur W. Galston

Morton H. Halperin
Garrett Hardin
Denis Hayes
William A. Higinbotham
John P. Holdren

Daniel Koshland, Jr.
Raphael Littauer
Franklin A. Long
Francis Low
Victor Rabinowitch

George W. Rathjens
Marc J. Roberts
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Frank Von Hippel
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Alfred Yankauer
Herbert F. York
*Nobel laureates

F. A. S.

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Washington, D.C. 20002 (202) 546-3300

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Secretary

Jerome D. Frank
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Herbert F. York
Treasurer

Jeremy J. Stor
Director

RESULTS OF DNA STRAW POLL FROM APRIL PUBLIC INTEREST REPORT

In its April publication, FAS discussed DNA recombinants and the problems which this kind of genetic engineering might produce. It asked members to read the Report and to advise whether the guidelines appeared to them to be reasonable. Members were asked to advise, also, whether they were or were not biologists.

As the table below reveals, 50% of non-biologists and 64% of the biologist respondents supported the guidelines. 30% of non-biologist respondents thought them insufficiently cautious, however, as did 25% of the biologists. Five to ten percent thought them probably too restrictive.

	<u>biologists</u>	<u>non-biologists</u>	<u>total</u>
A. "probably too restrictive"	12	11	23
B. "probably about right"	83	98	181
C. "probably insufficiently cautious"	32	61	93
D. "insufficient information"	2	23	25
	129	193	322

[546]

National Council Members (Elected)

Ruth S. Adams
David Baltimore
Nina Byers
Rose E. Frisch
Arthur W. Galston

Morton H. Halperin
Garrett Hardin
Denis Hayes
William A. Higinbotham
John P. Holdren

Daniel Koshland, Jr.
Raphael Littauer
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Marc J. Roberts
Leonard Rodberg
Joseph L. Sax
George A. Silver

Jeremy J. Stone
Vigdor Teplitz
Frank Von Hippel
Myron E. Wegman

May 28, 1976

Dr. Donald Fredrickson
Director, National Institutes of Health
Bethesda, Maryland 20014

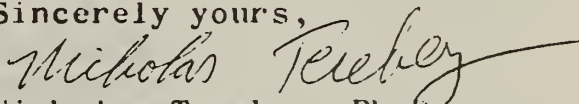
Dear Dr. Fredrickson:

I have lately taken a serious academic interest in the current controversy over the rules governing future experiments on genetic recombination via bacterial plasmids or viruses. Far from being trivial exercises, this problem assumes immense importance when serious scientists such as Dr. Paul Berg announce that they would be extremely interested in studying the behavior of nucleic acids taken from oncogenic viruses when such nucleic acids are joined to the genome of common organisms such as E. coli. If there are benefits to be gained in such research, are the risks worth it?

My opinion is that all such research should be banned. The only exception or condition under which this research could continue would be if proven methods of biological containment could be developed which would not depend on common organisms such as E. coli which normally comprise the human flora. Surely if E. coli can accept plasmids incorporating heterologous DNA, there must be other organisms which can do the same thing, grow just as well under laboratory conditions but not inhabit or be potentially pathogenic in humans. The actual problem may be that not enough research has been done to advance the practicality of biological containment. The description of these methods in the recent issue of ASM News was sketchy and speculative at best.

I hope my comments are of some interest.

Sincerely yours,


Nicholas Terebey, Ph.D.

209 E. 25th St.,
New York, New York 10010

Washtenaw County Democratic Party
235 Taft
Ypsilanti, Michigan 48197

May 26, 1976

The Honorable Philip Hart
Senate of the United States
Washington, D. C. 20510

MW
JUN 2 RECD

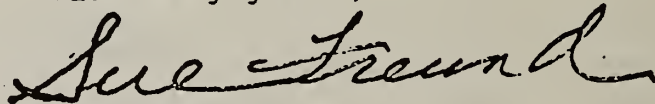
Dear Mr. Hart:

Enclosed please find a copy of the Resolution on Safeguards for Recombinant DNA Research, which the Washtenaw County Democratic Party adopted unanimously on May 20, 1976 and which the Party has asked me to forward to you.

In considering the resolution, members of the Party were guided by the extensive discussion of the research which has occurred locally over the past few months in the press and in symposia at the University of Michigan. They were also advised by a statement on the research which was prepared by a member who has a background in molecular biology. A copy of this statement is also enclosed. You may find it useful in your consideration of the issue addressed in the Resolution.

The issue of safeguards for recombinant DNA research is an important one that should be considered promptly by public officials at all levels of government. The research is already occurring at many places under restrictions far less stringent than those suggested in the Resolution. The longer the delay before adequate safeguards are imposed, the greater the likelihood becomes that people or the environment will be injured. The Party urges you to consider the issue addressed in the Resolution promptly and hopes that you will consider the Resolution itself favorably.

Sincerely yours,



Sue Freund
Chairperson

Enclosures

SF/ws

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Resolution on Safeguards for Recombinant DNA Research

The Washtenaw County Democratic Party is concerned that recombinant DNA research be regulated adequately to protect people and the environment from its potential dangers.

In particular, the Party recommends that the research be restricted by law to a limited number of facilities equipped to prevent the escape of organisms containing recombinant DNA, until lesser restrictions can be justified with sufficient reliable data on the magnitudes of the risks of the research and with the development of adequate safeguards based on such data.

Adopted May 20, 1976

STATEMENT ON RECOMBINANT DNA RESEARCH

Recombinant DNA research involves producing new organisms through combination of genetic material from organisms that do not normally interact genetically.

The research may lead to improvements in human health and welfare, but it entails risks of serious, possibly irreversible damage to people and the biosphere. Benefits foreseen from the research include extended knowledge of the organization and control mechanisms of genes, treatments and cures for diseases involving genetic malfunctions, improvements in agricultural productivity, new methods of pollution control, and methods to produce abundantly drugs that are now scarce. Dangers include release of new disease-causing or toxin-producing microorganisms, release of more virulent or antibiotic-resistant strains of disease-causing microorganisms already known, introduction into the biosphere of new organisms that upset ecological balances, development of new biological weapons for use in war and by terrorists, and misuse of abilities that may be developed to do human genetic engineering.

The dangers posed by recombinant DNA research require that it be done only with precautions adequate to safeguard people and the environment. The people of Washtenaw County are particularly concerned that there be such precautions. Already there are at least three institutions in the County at which recombinant DNA research could be carried out: the University of Michigan, Eastern Michigan University and Parke-Davis Research Laboratories. Elsewhere in Michigan there are several more institutions with capability to do the research. Recombinant DNA techniques are likely to become more widely known, easier to use and less expensive very rapidly; consequently the number of institutions at which they may be used will quickly increase. Appropriate legislation and regulations are needed now to protect the public from the possible deleterious consequences of recombinant DNA research.

When the research first became possible in 1972, scientists involved recognized its hazards. Their concern led them to impose a voluntary ban, that has been generally followed, on the forms of the research considered by them to be most hazardous. It also led to establishment of the Recombinant DNA Molecule Advisory Committee under the auspices of the National Institutes of Health (NIH). The NIH funds much of the genetic research done at non-profit organizations in the United States. The charge of the committee is to recommend to the NIH Director guidelines to govern the safe conduct of recombinant DNA research funded by the NIH.

The Director will shortly promulgate the first set of guidelines based on the committee's recommendations. Some scientists believe the recommendations, even if scrupulously followed by researchers, provide inadequate safeguards. Enforcement of the guidelines will be difficult enough for research funded by the NIH. They will not even apply to research not funded by the NIH, which includes some done at universities and all supported by industry and by the Department of Defense.

Most knowledgeable scientists agree that there is little data on the magnitude of the risks involved with recombinant DNA research. In the absence of such data, it is not possible to evaluate the adequacy of safeguards proposed for the research. In this situation, wisdom and safety require extreme caution, including restricting the research to locations where, as nearly as possible, surviving organisms containing recombinant DNA can be totally contained. Damage arising from inadequate safeguards may be irreversible. On the other hand, if reliable data on the hazards of the research are acquired to establish that lesser restrictions would suffice to safeguard people and the environment, restrictions can always be relaxed accordingly.

Rather than being permitted with virtually no constraints at an unlimited number of places, recombinant DNA research should be restricted through appropriate legislation and regulations to a few facilities which are equipped to prevent the escape of organisms containing recombinant DNA. There are now twenty-one such facilities in the United States.

Scientists at institutions which do not have such facilities would not be precluded from doing recombinant DNA research. Arrangements could easily be made, through the NIH or a multi-university consortium for example, for the sharing of such facilities so that scientists would have access to a facility when they need to use recombinant DNA techniques in their research. Similar arrangements already exist for some research in high energy physics and astronomy.

Bill Scanlon

PETITION ON RECOMBINANT DNA RESEARCH

To: Dr. Donald S. Frederickson, Director, NIH

We the undersigned are biologists who are deeply troubled by the potential dangers of recombinant DNA research. We do not advocate a complete moratorium, nor do we question the potential merits of this research. We do believe, however, that the consequences of rearranging the products of hundreds of millions of years of evolution are unknown, and that the proposed biohazard containment procedures and other guidelines are arbitrary.

It is our view that (1) the pending NIH guidelines have been based largely, although not exclusively, on the input of the practitioners themselves, (2) the issue of advised public consent has not been raised, and (3) the alleged benefits to medicine and agriculture are not so imminent that this form of research cannot be postponed until a more comprehensive and representative dialogue has taken place.

The impending release of the NIH guidelines (scheduled for June 21) marks the time at which the United States government officially sanctions a form of experimentation that may be of unprecedented danger in the history of science. We believe that widespread and well-founded concern is just now surfacing and will begin to crystallize over the summer, both within the scientific establishment and the public at large. As the executive officer who must bear full responsibility, we urge you at this extraordinary moment in history to withhold release of the NIH guidelines until at least October 1, 1976.

Respectfully,

David Shore Dept. of Microbiology & Molecular
Genetics, Harvard Medical School

Charles L. Dismore " " " "

Shirley Galanter " " " "

Marion L. Berne " " " "

Edith Binstock " " " "

Jeffrey A. Miller " " " "

Barry Wanner " " " "

Hiroshi Tawarayama " " " "

Jonathan Leckwith " " " "

David E. Tress " " " "

Received 6/14/76

HEW



NEWS

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Dr. William J. Gartland
(301) 496-7714

FOR RELEASE AT 12 NOON
June 23, 1976

Dr. DeWitt Stetten
(301) 496-1921

Guidelines for the conduct of experiments involving recombinant DNA molecules were released today by Dr. Donald S. Fredrickson, Director of the National Institutes of Health.

Dr. Fredrickson also released a document describing the factors he took into account in reaching his decision to issue the Guidelines.

The NIH Guidelines will, effective today, govern research at laboratories of the NIH and those of its grantees and contractors. They are also expected to be adopted by other laboratories throughout the United States and foreign countries.

The NIH has also undertaken an environmental impact assessment of these Guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969 (NEPA). The purpose of this assessment is to review the environmental effects, if any, of research that may be conducted under the Guidelines.

Recombinant DNA molecules result from recombination in the test-tube of segments of deoxyribonucleic acid, the material which determines the hereditary characteristics of all living cells. These techniques have a remarkable potential for furthering the understanding of fundamental biochemical processes in cells of lower and higher organisms, and promise to revolutionize molecular

(more)

[552]

biology. But the technology, which permits genetic information from very different organisms to be combined, also involves potential hazards which are difficult to evaluate. Therefore the research must proceed with considerable caution.

Medical advances to be expected through the use of this technology include the opportunity to explore the functioning of cells in complicated diseases. Understanding of a variety of hereditary defects may be significantly enhanced, and some may be able to be prevented or modified. In the future it may be possible to use this technology to produce in microorganisms medically important compounds for the treatment and control of disease,

There are risks in the new research as well as potential benefits. Microorganisms with transplanted genes--called "chimeras"--may prove hazardous to human or other forms of life. Thus special provisions are necessary for their containment.

The NIH Guidelines establish carefully controlled conditions for the conduct of experiments involving the production of such molecules and their insertion into organisms such as bacteria. These Guidelines replace the recommendations contained in the 1975 Summary Statement of the Asilomar Conference on Recombinant DNA Molecules. The latter would have permitted research under less strict conditions than the NIH Guidelines.

The chronology leading to the present Guidelines is described in detail in the NIH Director's decision document. In summary, scientists engaged in this research called, in 1974, for a moratorium on certain kinds of experiments until an international meeting could be convened to consider the potential hazards of recombinant DNA molecules. They also called upon the NIH to establish a committee to provide advice on recombinant DNA technology.

(more)

The international meeting was held at the Asilomar Conference Center, Pacific Grove, California, in February 1975. The consensus of this meeting was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate physical and biological barriers. The Asilomar Conference report also made interim assignments of the potential risks associated with different types of experiments. The NIH then assumed responsibility for translating the broadly based Asilomar recommendations into detailed guidelines for research.

Dr. Fredrickson reached his decision on the Guidelines after extensive scientific and public airing of the issues during the sixteen months which have elapsed since the Asilomar Conference. The issues were discussed at public meetings of the Recombinant DNA Molecule Program Advisory Committee (Recombinant Advisory Committee) and the Advisory Committee to the NIH Director. The Recombinant Advisory Committee extensively debated three different versions of the Guidelines during this period.

The Advisory Committee to the NIH Director, augmented with consultants representing law, ethics, consumer affairs and the environment, was asked to advise as to whether the proposed Guidelines balanced responsibility to protect the public with the potential benefits through the pursuit of new knowledge. The many different points of view expressed at this meeting were taken into consideration by the Director in making his decision. Dr. Fredrickson emphasized, however, that NIH will continue to consider issues raised by the scientific community and the public on this type of research.

(more)

The NIH Guidelines identify experiments which are not to be performed at the present time. For permissible experiments, the Guidelines define different levels of physical and biological containment, and classify containment criteria for different kinds of recombinant DNAs. Used in the combination specified in the Guidelines, these measures are designed to afford protection to workers and the environment while permitting this important line of research to proceed.

The NIH Guidelines also define the responsibilities of investigators, institutions where the research is conducted, and NIH staff and advisory committees.

Dr. Fredrickson noted that NIH recognizes a special obligation to disseminate the Guidelines as widely as possible. Accordingly, the Guidelines will be sent to all of the approximately 25,000 NIH grantees and contractors. Major professional societies which represent scientists working in this area will also be asked to endorse the Guidelines.

The Guidelines will be sent to medical and scientific journals. Dr. Fredrickson will ask the editors of these journals to request that investigators include a description of the physical and biological containment procedures used in any recombinant research they report on.

Dr. William Gartland, who will head the NIH Office of Recombinant DNA Activities, will leave today for Geneva to brief various international health and scientific organizations with responsibility in this area. He will meet with the World Health Organization Advisory Committee on Medical Research that will be reviewing this matter this week.

(more)

Dr. Gartland will also brief appropriate officials at the European Molecular Biology Organization and in Great Britain. The Guidelines have also been sent to all science attaches of foreign embassies located in Washington and to U. S. science attaches in our embassies in foreign countries.

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

June 23, 1976

See Attached List of Addressees

Dear

Enclosed is a copy of the NIH guidelines on recombinant DNA research and my decision which accompanies them.

The NIH Guidelines will govern research at laboratories of the NIH and those of its grantees and contractors. The NIH has also undertaken an environmental impact assessment of these Guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969. The purpose of this assessment is to review the environmental effects, if any, of research that may be conducted under the Guidelines.

Recombinant DNA molecules result from recombination in the test-tube of segments of deoxyribonucleic acid, the material which determines the hereditary characteristics of all living cells. These techniques have a remarkable potential for furthering the understanding of fundamental biochemical processes in cells of lower and higher organisms, and promise to revolutionize molecular biology. But the technology, which permits genetic information from very different organisms to be combined, also involves potential hazards which are difficult to evaluate. Therefore the research must proceed with considerable caution.

Medical advances to be expected through the use of this technology include the opportunity to explore the functioning of cells in complicated diseases. Understanding of a variety of hereditary defects may be significantly enhanced, and some may be able to be prevented or modified. In the future it may be possible to use this technology to produce in microorganisms medically important compounds for the treatment and control of disease.

There are risks in the new research as well as potential benefits. Microorganisms with transplanted genes--called "chimeras"--may prove hazardous to human or other forms of life. Thus special provisions are necessary for their containment.

The NIH Guidelines establish carefully controlled conditions for the conduct of experiments involving the production of such molecules and their insertion into organisms such as bacteria. These Guidelines

replace the recommendations contained in the 1975 Summary Statement of the Asilomar Conference on Recombinant DNA Molecules. The latter would have permitted research under less strict conditions than the NIH Guidelines.

The chronology leading to the present Guidelines is described in detail in my decision document. In summary, scientists engaged in this research called, in 1974, for a moratorium on certain kinds of experiments pending an assessment of potential hazards of this research and the development of appropriate guidelines. In response, the National Institutes of Health and the National Science Foundation supported a conference sponsored by the National Academy of Sciences which was held at the Asilomar Conference Center in California in February 1975. The consensus of the meeting at Asilomar was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate precautions. The Asilomar Conference report also made interim assessments of the potential risks associated with different types of experiments.

The Asilomar Conference actions led the NIH to establish an advisory committee to develop guidelines for recombinant research funded or conducted by the NIH and to devise programs for assessing and controlling hazards in such research. After a year's work, the committee in December 1975 proposed guidelines for the NIH to govern such DNA recombinant research.

The proposed guidelines were reviewed at a special meeting of the Director's Advisory Committee held at the NIH on February 9-10, 1976. Members of the committee represented areas including not only science, but also law, ethics and consumer affairs. The meeting afforded an opportunity for the scientific community and the public to comment on the proposed guidelines. Over the past several months I reviewed the proposed guidelines in the light of the comments and suggestions made by the participants at the meeting as well as the written comments received after the meeting. A number of issues of special concern to the commentators were reviewed at my request by the advisory committee that developed the guidelines. I considered carefully the responses of that committee to the issues raised by the commentators.

Within the month the NIH will publish a report containing the full transcript of the public hearing, the statements filed by public and scientific witnesses and the correspondence addressed to me on this matter. My decision, which is based on that record, examines each of the substantive issues presented with an explanation of my decision on each issue. These guidelines contain a number of revisions based on that analysis. On the basis of continuing discussions with other organizations which are conducting, monitoring or supporting this type

Page 3 -

of research in this country and abroad, we believe these guidelines will be adopted by other laboratories throughout the United States and that they will provide a basis for development of similar guidelines in foreign countries.

If you have any questions or comments on the guidelines or the issues raised in my decision paper, I would be pleased to respond to them.

Thank you very much.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Enclosures

ADDRESSEES OF LETTER TO CONGRESSIONAL COMMITTEES

U.S. Senate

The Honorable Edward M. Kennedy
Chairman, Subcommittee on Health
Committee on Labor and Public Welfare

The Honorable Jacob K. Javits
Subcommittee on Health
Senate Committee on Labor and Public Welfare

The Honorable Richard S. Schweiker
Subcommittee on Health
Senate Committee on Labor and Public Welfare

The Honorable Warren G. Magnuson
Chairman, Subcommittee on Labor-HEW Appropriations
Committee on Appropriations

The Honorable Edward W. Brooke
Subcommittee on Labor-HEW Appropriations
Committee on Appropriations

House of Representatives

The Honorable Paul G. Rogers
Chairman, Subcommittee on Health and the Environment
Committee on Interstate and Foreign Commerce

The Honorable Tim Lee Carter
Subcommittee on Health and the Environment
Committee on Interstate and Foreign Commerce

The Honorable Daniel J. Flood
Chairman, Subcommittee on Labor-HEW Appropriations
Committee on Appropriations

The Honorable Robert H. Michel
Subcommittee on Labor-HEW Appropriations
Committee on Appropriations

Others who received copies of the NIH Guidelines, though not with the above letter:

The Honorable Mark O. Hatfield
Committee on Appropriations
U.S. Senate

The Honorable Thomas P. O'Neill, Jr.
Majority Leader
House of Representatives



[Copies sent on June 23, 1976, to countries believed to have a substantial biomedical research capability.]

Department of State

TELEGRAM

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PAGE 01 STATE 154658

13
ORIGIN HEW-06

INFO OCT-01 AF-08 ARA-10 EA-09 EUR-12 NEA-10 IO-13 ISO-00
OES-06 SIG-02 ERDA-07 /084 R

DRAFTED BY HEW/NIH:JRQUINN
APPROVED BY OES/APT/BMP:WJWALSH, III
NEA/EX:EGABINGTON(INFO)
ARA/EX:GECHAFIN(INFO)
EA/EX:JEFFREY CUNNINGHAM(INFO)
EUR/EX:CEREDMAN(INFO)
AF/EX:TFORD(INFO)
DHEW/OIH-JR KING

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[562]



Department of State **TELEGRAM**

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PAGE 02 STATE 154658

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USMISSION USUN NEW YORK
USMISSION OECD PARIS
AMCONSUL CAPE TOWN

UNCLAS STATE 154658

CAPE TOWN FOR EMBASSY

E.O. 11652: N/A

TAGS. TPHY, OSCI, TBIO, XA, XB, XM, XF

SUBJECT: NIH GUIDELINES ON DNA/RNA(GENETIC ENGINEERING ETC.) RECOMBINANT RESEARCH

1. NATIONAL INSTITUTES OF HEALTH PLANS ANNOUNCE GUIDELINES ON JUNE 23, 1976, CONCERNING RECOMBINANT DEOXYRIBO NUCLEIC ACID(DNA) RESEARCH DEVELOPED AS A RESULT OF OPEN DISCUSSIONS WITH U.S. SCIENTISTS.

2. IN VIEW OF SERIOUS IMPORTANCE OF SUBJECT TO WORLD'S SCIENTISTS AND NEED FOR WORLD PUBLICITY AND COOPERATION ON PROBLEM, DEPARTMENT REQUESTS ADDRESSEE MISSIONS ALERT

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[563]



Department of State

TELEGRAM

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PAGE 03 STATE 154658

APPROPRIATE OFFICIALS OF HOST GOVERNMENT'S AGENCY MOST CONCERNED WITH SCIENCE AND/OR SUPPORT OF BIOMEDICAL RESEARCH AND OFFER TO PROVIDE COPY OF GUIDELINES SOONEST. COMMENTS ON GUIDELINES MAY BE ADDRESSED TO DIRECTOR, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND 20014.

3. TWO COPIES OF THE GUIDELINES WILL BE AIRPOUCHED SEPARATELY TO POST SHORTLY AFTER RELEASE DATE OF JUNE 23. APPRECIATE DELIVERY OF GUIDELINES ASAP.

4. NIH ALSO PLANS SEND COPY GUIDELINES DIRECTLY TO ALL GRANTEES AND CONTRACTORS, DOMESTIC AND FOREIGN.

5. PLEASE NOTIFY NIH (ATTN: DR. J.R. QUINN) OF PERSON IN HOST GOVERNMENT ADVISED OF ABOVE MESSAGE AND TO WHOM GUIDELINES DELIVERED. ROBINSON

UNCLASSIFIED

[564]



[Sample letter to U.S. Ambassadors abroad]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

June 23, 1976

Dear Mr. Ambassador:

We are addressing this letter to you in order to assure that the important documents attached hereto are delivered to the most appropriate biomedical research agency of your host government. Where a science attache has been assigned to a post the letter has been addressed directly to him. However, in the absence of a science attache, would you kindly assign a member of your staff responsibility for assuring that the documents are delivered to the most appropriate senior official of the host government's agency responsible for promoting and supporting biomedical research. If such an agency does not exist, perhaps it could be delivered to a senior official of the appropriate national science agency.

We would also appreciate receiving by telegram the name and title of the person to whom the guidelines have been delivered.

We deeply appreciate your assistance in this matter.

Sincerely yours,

/s/

Milo D. Leavitt, Jr., M.D.
Director
Fogarty International Center

Attachments

[565]



[Sample letter to U.S. Scientific Attaches abroad]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

BETHESDA, MARYLAND 20014

June 23, 1976

Dear

On June 18, 1976, NIH sent a draft telegram to the Department of State announcing the publication of NIH guidelines on recombinant deoxyribonucleic acid (DNA) research to investigators supported by NIH funds under grants and contracts.

There are attached two copies of the guidelines, as well as the Decision of the Director, NIH, to Release the Guidelines, for delivery to the most appropriate biomedical research agency for the host government. Comments on the guidelines may be addressed directly to Dr. Donald S. Fredrickson, Director, National Institutes of Health, Bethesda, Maryland 20014.

Would you also please let us know by telegram to whom you delivered the statement and guidelines.

With deep appreciation,

Sincerely yours,

/s/

Milo D. Leavitt, Jr., M.D.
Director
Fogarty International Center



[Sample letter to Scientific Representatives at foreign embassies in Washington.]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

June 23, 1976

Dear

On June 23, 1976 the National Institutes of Health plans to release its guidelines on recombinant deoxyribonucleic acid (DNA) research to investigators supported by NIH funds under grants and contracts.

A copy of the guidelines is attached for your information and delivery to the appropriate biomedical research agency of your government. Comments on the guidelines may be addressed directly to me.

At present, there are only a limited number of copies available. However, additional copies will be available at a later date.

Your cooperation in bringing this to the attention of your government is appreciated.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Attachment

ADDRESSEES OF JUNE 23, 1976, LETTER TO FOREIGN EMBASSIES IN
WASHINGTON, D.C., WHICH HAVE A SCIENTIFIC REPRESENTATIVE

Dr. L. Groven
Embassy of Belgium

Dr. Yu Sung
Chinese Embassy

Mr. E. Rahikainen
Embassy of Finland

Dr. K. Windieck
Embassy of the Federal
Republic of Germany

Dr. Edward G. Bowen
Embassy of Australia

Dr. M. Anandakrishnan
Embassy of India

Mr. L. Ortegren
Royal Swedish Embassy

Dr. Y. N. Gubkin
Embassy of the U.S.S.R.

Mr. E. Efrati
Embassy of Israel

Dr. Y. Lim
Embassy of Korea

Mr. C. H. van Vierssen
Embassy of the
Netherlands

Dr. J. McDowall
Embassy of Canada

Prof. J. Jarosch
Embassy of Czechoslovak
Socialist Republic

Mr. Francois Davoine
Embassy of France

Dr. H. Lange
Embassy of the German
Democratic Republic

Mr. A. Smith
British Embassy

Dr. R. Shuttleworth
Embassy of South Africa

Dr. C. Favre
Embassy of Switzerland

Mr. M. Rajacic
Embassy of the Socialist
Federal Republic of
Yugoslavia

Mr. K. Nagara
Embassy of Japan

Dr. E. De Alba
Embassy of Mexico

Mr. R. Wojnicki
Embassy of Polish
People's Republic

PRESS CONFERENCE ON DNA GUIDELINES

June 23, 1976

OPENING REMARKS

By

DONALD S. FREDRICKSON, M.D. 1/

Today, with the concurrence of the Secretary of Health, Education, and Welfare, and the Assistant Secretary for Health, I am issuing guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules. The NIH has also undertaken an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969. The purpose of this assessment will be to review the environmental effects, if any, of research that may be conducted under the guidelines. The assessment will provide further opportunity for all concerned to address the potential benefits and potential hazards of this important research activity.

I expect a draft of the environmental impact statement to be completed by September 1 for comment by the scientific community, Federal and state agencies, and the general public.

1/ Director, National Institutes of Health, Bethesda, Maryland

These guidelines replace the guidelines issued in 1975 in the Summary Statement of the Asilomar Conference on Recombinant DNA Molecules.

The experiments voluntarily deferred under the Asilomar guidelines are still prohibited by the NIH guidelines. The Asilomar guidelines in many instances permitted certain experiments to proceed under less strict conditions than do the NIH guidelines. The latter define much more explicitly the physical and biological containment conditions designed to protect workers and the environment, while permitting this important line of work to proceed.

The NIH guidelines contain many details concerning safety practices and define the responsibilities of investigators and institutions where the research is to be conducted. Finally, a structure is established for implementation by NIH staff and advisory committees.

Thus, these guidelines are being released prior to the completion of the environmental impact assessment, because they will provide still greater protection for the public and the environment than the current Asilomar guidelines might afford. The NIH guidelines will be in effect while the environmental impact assessment is under way.

As most of you know, approximately two years ago, scientists engaged in recombinant DNA research voluntarily called for a moratorium of certain experiments pending an assessment of potential hazards of this research and the development of appropriate guidelines. In response, the National Institutes of Health and the National Science Foundation supported a

conference sponsored by the National Academy of Sciences which was held at the Asilomar Conference Center in California in February 1975. The consensus of the meeting at Asilomar was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate precautions. The Asilomar Conference report also made interim assessments of the potential risks associated with different types of experiments.

The Asilomar Conference actions led the NIH to establish an advisory committee to develop guidelines for recombinant research funded or conducted by the NIH and to devise programs for assessing and controlling hazards in such research. After a year's work, the committee in December 1975 proposed guidelines for the NIH to govern such DNA recombinant research.

The proposed guidelines were reviewed at a special meeting of the Director's Advisory Committee held at the NIH on February 9-10, 1976. Members of the committee represented areas including not only science, but also law, ethics and consumer affairs. The meeting afforded an opportunity for the scientific community and the public to comment on the proposed guidelines. Over the past several months I reviewed the proposed guidelines in the light of the comments and suggestions made by the participants at the meeting as well as the written comments received after the meeting. A number of issues of special concern to the commentators were reviewed at my request by the advisory committee that developed the guidelines.

I considered carefully the responses of that committee to the issues raised by the commentators. The NIH will publish a report containing the full transcript of the public hearing, the statements filed by public and scientific witnesses and the correspondence addressed to me on this matter. My decision, which is based on that record, examines each of the substantive issues presented with an explanation of my decision on each issue. The guidelines being released today contain a number of revisions based on that analysis.

The NIH guidelines will govern research at laboratories of the NIH and those of its grantees and contractors. On the basis of continuing discussions with other organizations which are conducting, monitoring or supporting this type of research in this country and abroad, we believe these guidelines will be adopted by other laboratories throughout the United States and that they will provide a base for development of similar guidelines in foreign countries.

The NIH recognizes a special obligation to disseminate information on these guidelines as widely as possible. They, and the decision paper accompanying their release, will be published forthwith in the Federal Register for comment. Copies will be distributed to all who have contributed to debate concerning them. Filing of the environmental impact statement will provide further opportunity for the scientific community, Federal, State and local agencies and the general public to address the potential benefits and hazards of this research area.

Beginning tomorrow Dr. Gartland will also be briefing appropriate officials at the World Health Organization, The European Molecular Biology Organization and in Britian. Today the guidelines will also be sent to all science attaches of foreign embassies located in Washington and to U.S. science attaches in our embassies in foreign countries.

These NIH guidelines for conduct of recombinant DNA research represent a small but important step in developing standards and criteria to protect the public interest and to guide the decision-making process required for conservative exploitation of new knowledge that combines profound promise with uncertain hazards.

It is my belief that these guidelines, and the provision of all the information leading to their development and promulgation, constitute a new structure that will usefully serve to improve the quality of public debate and scientific consideration of this exceedingly important matter.

WASHINGTON STAR
Washington, D.C., 6/23/76

NIH Issues Guidelines For Genetic Research

By Cristine Russell
Washington Star Staff Writer

The National Institutes of Health director today issued new guidelines to strengthen safeguards on genetic research of the sort that could "create novel forms of life with potentially disastrous consequences."

Taking into account the "concerns of those who urge that this research proceed because of the benefits and of those who urge caution because of potential hazards," the NIH director, Dr. Donald S. Fredrickson issued guidelines which he said would "allow the research to go forward in a manner responsive and appropriate to hazards that may be realized in the future."

The guidelines established "carefully controlled conditions" for conducting specific genetic research which would provide an even "greater degree of scrutiny and protection" than general measures earlier recommended by an international gathering of scientific experts.

The guidelines would continue a prohibition on experiments considered too dangerous to be carried out at this time and would impose various combinations of physical and biological containment to provide maximum experimental safety.

THE RESEARCH in question technically known as "recombinant DNA research" involves cutting and splicing pieces of genetic material from different organisms and inserting the new combination into bacterial cells.

Potential benefits from such scientific tampering include revolutionary advances in understanding disease processes and, ultimately, practical boosts to medicine and agriculture. But treading into the unknown also carries speculative risks that harmful micro-organisms carrying pathogenic or cancer-causing genes or increased drug resistance to antibiotic drugs may possibly be created.

Fredrickson's long awaited announcement follows a period of unprecedented self-restraint since scientists themselves called attention to potential hazards in their research three years ago.

SCIENTISTS FROM around the world met in Asilomar, Calif., 16 months ago to draw up general genetic research safety guidelines. Since then, pending a more explicit directive from the NIH, major source of federal biomedical research support, U.S. recombinant DNA research has slowed to a snail's pace, except in that involving minimal risk

With today's announcement, the research pace is expected to pick up, but debate about the nature of the risks involved in conducting this controversial research

— and the degree of protection the guidelines provide — is likely to continue.

Tonight the Cambridge City Council will hold a hearing to consider the potential hazards of Harvard University's planned construction of a special containment laboratory for conducting such research. Cambridge Mayor Alfred E. Zelucci has already expressed his reservations, saying, "We may make a creature that slips beyond control and turns around to destroy the creator."

NIH WILL SEND two representatives to Cambridge to hand-carry copies of the new guidelines and explain them to the evening gathering.

Dr. Maxine Singer, one of the envoys, said yesterday she is firmly convinced the guidelines "offer a high degree of protection."

Dr. Dewitt Stetten, NIH's deputy director for science, who has carefully shepherded the recombinant DNA review, emphasized in an interview that the new guidelines are "far more specific and far more stringent" than the guidelines first outlined at the Asilomar conference.

"Any notion that we're opening the flood gates is quite wrong. In effect we're closing potential leaks," he said.

To allow public comment, the guidelines will be published in the Federal Register, and NIH has also undertaken an environmental impact assessment of them in compliance with the National Environmental Policy Act. The document is expected to be completed by September 1, providing further opportunity for the public to participate and comment on the research.

ARKANSAS GAZETTE
Little Rock, Ark., 6/24/76

Rules Issued Restricting DNA Research

DULUTH HERALD
Duluth, Minn., 6/23/76

Gene research rules released

ASSOCIATED PRESS
Washington, D.C., 6/23/76

NO80

R
DNA GUIDELINES
BY WARREN E. LEARY

WASHINGTON (AP) -- WHILE THE REAL POTENTIAL HEALTH HAZARD OF A CONTROVERSIAL KIND OF GENETIC RESEARCH CANNOT BE DEFINED WITH PRESENT KNOWLEDGE, THE DIRECTOR OF THE NATIONAL INSTITUTES OF HEALTH SAYS COMPLIANCE WITH NEW GUIDELINES SHOULD GREATLY CUT THE RISKS.

DR. DONALD S. FREDRICKSON SAID THE NIH GUIDELINES ISSUED WEDNESDAY FOR RECOMBINANT DNA RESEARCH "SHOULD HELP THOSE INVOLVED IN THE DEBATE OVER THIS TO CLARIFY THEIR ARGUMENTS."

FREDRICKSON SAID THE "GENERALLY CONSERVATIVE GUIDELINES" WOULD BAN SOME POTENTIALLY DANGEROUS WORK AND SET UP MORE RIGID STANDARDS THAN PRESENTLY USED TO REDUCE THE RISK OF ACCIDENTS THAT COULD RELEASE DANGEROUS ORGANISMS INTO THE ENVIRONMENT.

DNA -- DEOXYRIBONUCLEIC ACID -- IS THE CHEMICAL FORMING GENES, THE BASIC UNITS OF HEREDITY. RECOMBINANT DNA MOLECULES RESULT FROM RECOMBINING IN A TEST TUBE DNA FROM DIFFERENT SPECIES OF LIFE.

BACTERIA MODIFIED WITH RECOMBINANT DNA REPRODUCE AND HAVE OFFSPRING WITH CHARACTERISTICS OF THE OTHER SPECIES.

SUPPORTERS SAY THE RESEARCH HAS VAST POTENTIAL FOR SCIENTIFIC BREAKTHROUGHS. FOR EXAMPLE, THEY SEE TURNING BACTERIA INTO LITTLE FACTORIES PRODUCING VALUABLE PROTEINS AND HORMONES, SUCH AS INSULIN AND BLOOD CLOTTING FACTOR.

CRITICS SAY THE RESEARCH CAN CREATE SUPER DISEASE ORGANISMS NOT FOUND IN NATURE THAT ARE IMMUNE TO ALL KNOWN DEFENSES AND TREATMENTS.

THE NEW GUIDELINES BAN RESEARCH COMBINING GENES FROM CERTAIN RISKY DISEASE ORGANISMS INTO OTHERS AND PROHIBIT TRANSFERRING DRUG-RESISTANT TRAITS TO MICROORGANISMS THAT COULD COMPROMISE USE OF A DRUG IN CONTROLLING DISEASE.

THE GUIDELINES GO INTO GREAT DETAIL DESCRIBING FOUR CLASSES OF LABORATORIES IN WHICH THE RESEARCH CAN BE DONE.

DEPENDING UPON THE GENETIC MATERIAL USED AND THE EXPERIMENTAL OBJECTIVES, THE LABORATORIES CAN RANGE FROM ONES SIMILAR TO COMMON COLLEGE LABS TO OTHERS WITH ELABORATE SYSTEMS OF AIRLOCKS AND FILTERS RESEMBLING SPACE CAPSULES.

FREDRICKSON SAID THE GUIDELINES ARE EFFECTIVE IMMEDIATELY AT NIH LABORATORIES AND THOSE OF ITS CONTRACTORS AND GRANTEEES. NIH CURRENTLY SUPPORTS ABOUT \$4 MILLION WORTH OF THIS RESEARCH, HE ADDED.

THE GUIDELINES ARE NOT MANDATORY FOR RESEARCH OUTSIDE NIH, BUT FREDRICKSON SAID IT IS HOPED ALL DOING THIS TYPE OF WORK, INCLUDING PRIVATE INDUSTRY, WILL COMPLY VOLUNTARILY.

INTERNATIONAL HEALTH AND SCIENTIFIC ORGANIZATIONS HAVE BEEN AWAITING THE GUIDELINES, SAID NIH OFFICIALS, AND SEVERAL INDICATE THEY PLAN TO ADOPT THEM IN OTHER COUNTRIES.

THE GUIDELINES REPLACE INFORMAL RECOMMENDATIONS DRAWN UP LAST YEAR BY AN INTERNATIONAL GROUP OF SCIENTISTS ON HOW TO CONDUCT THE RESEARCH AND ON WHAT WORK SHOULD NOT TAKE PLACE. FREDRICKSON SAID MOST SCIENTISTS WELCOMED THE GUIDELINES.

"THE GREATEST FEAR OF THE SCIENTIFIC COMMUNITY IS THAT THERE IS TREMENDOUS POTENTIAL POWER IN THIS TECHNIQUE AND THEY WANT TO BE VERY CONSERVATIVE ABOUT USING IT," HE SAID.

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BEE
Modesto, Calif., 6/24/76

Guidelines for genetic research

Issue Some Rules on Making New Genes Out of Old

By BARBARA YUNCKER

The federal government today put strict limits, effective immediately, on what kinds of genetic research can be done in government medical research laboratories and outside laboratories which receive federal grants.

The new rules will prohibit putting genes together from different species of bacteria if risky disease organisms are involved; if the combination were to insert drug resistance into a disease microbe or if the combined

genetic material would have the possibility of creating potent poisons.

The regulations were issued today in Bethesda, Md., by Dr. Donald S. Fredrickson, director of the National Institutes of Health, the medical research arm of the Dept. of Health, Education and Welfare.

Fredrickson said the rules apply specifically to the laboratories at NIH and to its grantees and contract holders. But it is expected that

they will be adopted generally throughout the U.S. and abroad, he said.

The rules are to control an area of study called recombinant DNA research. DNA—deoxyribonucleic acid—is the basic molecule of living material and in its various forms it determines all the individual characteristics of every species, as passed on through heredity.

Scientists are now learning to take segments of DNA from one species of living organism—so far only bac-

teria which are single-celled—and combine it in the test tube with DNA from another species and thus create a new form of bacterial life.

It is this potential which has raised enormous ethical concern in the scientific community, with some members calling for a halt on all such genetic tinkering.

The guide lines issued today stop short of a total ban on DNA recombinations, but go further than NIH had originally indicated it would do.

The regulations, in addition to banning some experiments altogether, limit to 10 liters the cultures of recombinants which are known to have the capacity to create harm.

The new rules will put a damper on an area of research which holds out tremendous promise as well as possible perils.

The technology offers unparalleled promise of deciphering the mechanisms of a variety of hereditary defects.

Continued on Page 18

NEW YORK TIMES
New York, N.Y., 6/24/76

Guidelines Issued to Curb Genetic Research Hazards

By HAROLD M. SCHMECK Jr.

Special to The New York Times

WASHINGTON, June 23—others, graded safety precautions to govern revolutionary and potentially hazardous genetics research were issued today by the National Institutes of Health for distribution to American scientists and to scientific societies and governments throughout the world.

The research in question has been called one of the most exciting frontiers of biological science. Some aspects of it, however, have been deemed so potentially dangerous to public health that scientists adopted a moratorium on some experiments.

Certain experiments are prohibited for the present. For

Continued on Page 30, Column 6

Guidelines Issued on Genetic Research Hazards

Continued From Page 1, Col. 3

be sterilized before removal; the ventilation system would filter all outgoing air and keep the laboratories at lower pressure than the outside so that no unfiltered air could escape.

The guidelines made public today are the culmination of about three years of intense debate among scientists and some laymen.

At a news conference today, Dr. Donald S. Fredrickson, director of the N.I.H., said that several countries were considering national guidelines to govern research of the same sort and that these were likely to follow closely the guidelines promulgated today.

The scientific studies and experiments that have drawn such great international interest in recent years are generally called recombinant DNA research. In these experiments pieces of the genetic material DNA (deoxyribonucleic acid) from different species are joined together and transplanted into living cells. With this kind of recombination and transplantation, genes of plants, animals or even man could be grown in bacteria.

Potentially a living organism such as a bacterium could be given traits and capabilities that it never has in nature.

Dr. Fredrickson said that the techniques were of tremendous potential power and that scientists wanted to use that power in an extremely conservative fashion.

On the positive side, scientists have suggested that such research might revolutionize agriculture, the production of drugs and the understanding of many baffling diseases. On the negative side, recombinant DNA experiments might conceivably loose on the world more deadly and difficult to control than any produced in nature.

It might, for example, prove to be only 27 laboratories in possible to transplant into com- the United States capable of mon bacteria the human genes meeting the standards for the that govern production of the highest security level in the hormone insulin. If successful, guidelines. The cost of building this could tremendously simplify a new laboratory of this fy the industrial production of sort was estimated at about insulin for diabetics. \$750,000 by an expert at the

If some of these hypothetical insulins-producing bacteria escaped from the laboratory, however, and infected men and animals, they might do unpredictable global harm.

In answer to questions at the news conference today, Dr. Fredrickson said it was possible to imagine situations involving all manner of potential dangers from recombinant DNA work, but it was not possible now to estimate their probability of occurring, if indeed there was any probability at all.

Moratorium Supplanted
The guidelines supplant the moratorium established by the scientists themselves about two years ago and interim recommendations for the research drafted last year at a meeting of scientists in California. The

new guidelines are somewhat more strict and considerably more detailed and specific than the earlier interim recommendations, according to scientists at the N.I.H.

At present, there are believed support of biomedical research,

The guidelines have purposely been kept flexible and will be revised when new knowledge warrants changes, Dr. Fredrickson said.

Among experiments barred those that would transplant genes for the production of powerful poisons into common and now harmless bacteria, experiments that would make dangerous germs resistant to the drugs currently used against them and deliberately released into the environment of any organism containing a recombinant DNA molecule.

The guidelines generally limit experimental production of recombinant material to small amounts so as to minimize risk of contamination outside the laboratories.

The new guidelines will apply to all research in this field supported by the N.I.H., which is the Federal Government's main agency for the conduct and support of biomedical research.

Rules Are Issued For DNA Labs

Continued from Page 5
and the possibility of controlling them through more sophisticated genetic manipulation than the bacterial work now going on.

The technique also offers the potential of turning bacteria into tailor-made generators of proteins and hormones which could be used to treat metabolic diseases.

The new guidelines provide for continuation of experiments with these goals if they can meet the standards for safety.

The fear is that such recombinants could devastate whole plant or animal populations if they were to proliferate beyond the laboratories and species exposed to them had no resistance.

The NIH also announced that it was undertaking an assessment of environmental impact by the kinds of research its rules will permit to continue—under the National Environmental Policy Act of 1969.

APPLETON POST-CRESCENT
Appleton, Wis., 6/23/76

Guidelines issued to try to keep genetic tests safe

Genetic Research Rules Issued

WASHINGTON (UPI)—The National Institutes of Health issued Wednesday guidelines designed to ensure that genetic research does not unleash new diseases or drug-resistant germs.

The guidelines, expected to be adopted worldwide, set up strict biological safety controls for laboratory recombinations of DNA (deoxyribonucleic acid), which determines the hereditary characteristics of all living cells.

The institutes' director, Dr. Donald Fredrickson, said, "The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public or on the environment."

More Restrictive

He said the guidelines ban certain research and generally are more restrictive than those urged by leading scientists in 1975.

The guidelines will enable research to continue on projects that have great potential value to medicine, science and technology, Fredrickson said in an unusual public document outlining the factors he considered in reaching his decision.

One potentially valuable line

of research, for instance, looks to development of bacteria to eat oil spills.

"The guidelines issued today allow the research to go forward in a manner responsive and appropriate to hazards that may be realized in the future," he said.

He said it was "extremely

important for the public to be aware that this research is not directed to altering of genes in humans, although some of the techniques developed in this research may have relevance if this is attempted in the future."

Fredrickson said the guidelines were developed with advice from consultants in the

fields of law, ethics, consumer affairs and the environment.

They will govern the institute's research, but their application is expected to be worldwide.

Copies are being sent to 25,000 grantees and contractors, medical and scientific journals, science attaches of em-

bassies in Washington and U.S. science attaches in foreign countries.

Dr. William Gartland, who will head an Institute Office of Recombinant DNA Activities, planned a trip to Geneva to brief international health and scientific organizations on the new recommendations.

COURIER-POST
Camden, N.J., 6/23/76

U. S. issues first guidelines for genetic experimentation

By JAMES ASHER
Courier-Post Staff

The National Institutes of Health, Bethesda, Md., issued guidelines today, forged during two years of controversy and debate, that establish limits for genetic experiments.

The scientific community, armed with these strict but voluntary guidelines, can resume research that could ultimately bypass evolution and create new forms of life.

Scientists, who have traditionally studied nature as they observed it, are now able to change the structure of molecules thereby creating new life forms.

When news of the scientific technique was disclosed in 1974, researchers agreed to a moratorium on experimentation to avoid mistakes that could create unstoppable epidemics spread by mutated bacteria.

The new guidelines suggest laboratory conditions that should eliminate the chance of accidental spread of mutated organisms.

Basically, scientists can take DNA, the building block of life, and cut it up with special enzymes.

By rearranging the DNA of certain organisms, scientists are hoping to create life forms unlike any others found in nature.

Far in the future, researchers hope to produce plants that contain nitrogen-producing bacteria that would eliminate the need for fertilizer, or animals resistant to disease.

The controversy, naturally enough, has come from scientists who are fearful about tampering with evolution.

Erwin Chargaff of Columbia Uni-

versity asks: "Have we the right to counteract, irreversibly, the evolutionary wisdom of millions of years in order to satisfy the ambition and curiosity of a few scientists?"

In a letter to *Science* magazine, Chargaff called this evolutionary alteration a crime and said: "It is so unthinkable to previous generations, that I could only wish that mutants were not guilty of it."

William Gartland, a member of the NIH committee that prepared the guidelines, said the issue has basically been resolved within the scientific community.

However, the guidelines are soon to be published in the Federal Register, the government's magazine listing all official business.

Once published, Gartland said, the NIH may be hearing from non-scientists raising issues of a non-scientific nature, thereby causing a new debate.

Not all the details of the guidelines were immediately available.

One of the prime criticisms about genetic research involves the host organism to be used when breaking apart the DNA.

Scientists will be using a bacteria found in the human gut and nose, *Escherichia coli*.

Critics contend that such *E. coli* bacteria, because of its viability within the human body, can spread unknown diseases after contact with the altered DNA.

Proponents challenge that, saying, the bacteria would not be able to live outside the laboratory.

To handle that problem, the NIH guidelines require that bacteria be weakened to lessen the likelihood of such infection. Also, NIH suggests

that no organisms be released into the environment.

Gartland said Tuesday the prohibition on release would apply even to strains of plants like those envisioned with nitrogen producing capacities. He said the guidelines may be altered to allow widespread cultivation.

The guidelines also suggest that scientists search for another host bacteria, one that does not have viability in humans.

Gartland doubted such an organism could be found soon.

He also noted the guidelines do not have the force of regulations but instead require the voluntary compliance of researchers.

WALL STREET JOURNAL
New York, N.Y., 6/24/76

Safety Guides on Genetic Experiments Issued by U.S. for Grantees, Contractors

By a WALL STREET JOURNAL Staff Reporter

WASHINGTON — The government took the first steps to control laboratory experiments in genetic manipulation of bacteria and other simple forms of life.

These experiments, being conducted in laboratories around the world, are aimed at bettering scientific understanding of DNA, the hereditary material of all living organisms. They could lead eventually to new methods of treating genetic diseases and the creation of improved varieties of agricultural crops. But genetic experiments also pose the threat of loosing on civilization lethal new germs manufactured in the laboratory.

The National Institutes of Health, the federal government's medical research arm, yesterday published the first set of guidelines, ruling out certain highly risky experiments and establishing safety standards for others.

Guidelines' Applications

The NIH guidelines will apply directly, only to the agency's 25,000 grantees and contractors, which include most of the nation's universities. But the guidelines are likely to be adopted in the same or only slightly modified form by the Pharmaceutical Manufacturers Association, representing most drug companies, which also engage in genetic experiments.

U.S. officials also hope the NIH guidelines, the first adopted by any nation, will set the standard for world-wide limitations on genetic experiments. It's likely U.S. policy will be emulated by other countries, but there isn't any guarantee.

The NIH guidelines, which take effect immediately, are based on months of debate in scientific circles. The move to limit genetic experiments was initiated by researchers in the field who worried that serious harm to their work and society would eventually arise if there weren't any safety standards.

Prohibited Experiments
Some biologists liken their dilemma to the difficulties faced by physicists at the dawn of the atomic age. They are in possession of a revolutionary new technology that promises great benefits to mankind but also poses immense dangers.

The NIH guidelines, for example, prohibit experiments that increase the resist-

ance of bacteria to drugs used in the treatment of human and animal disease. They also prohibit experiments with genetic material from organisms that cause human disease and the deliberate release of any organisms containing manufactured genetic material.

Allowable genetic experiments are classified according to their potential hazard. Standards are established for the safety facilities in which the experiments must be conducted, the most dangerous experiments requiring the most secure containment facilities.

The genetic experiments are conducted by use of chemicals called restriction enzymes, which snip out tiny segments of the DNA, or genetic material, of one organism so that it can be transferred to another. Thus, in theory, genes to prevent disease or to produce certain characteristics could be implanted in the cells of humans or other living organisms.

One reason for the rush to control these experiments is that the work isn't expensive and doesn't require highly sophisticated laboratory training. Scientists say a good high school student could probably perform genetic experiments in his own basement if he had the required chemicals.

NEWSDAY
Long Island, N.Y., 6/24/76

Creation-of-Life Rules

STAR
Minneapolis, Minn., 6/23/76

Guidelines ban some 'perilous' genetic research

Scientists Back Rules For Genetic Research

■ FROM PAGE ONE

ments in which the genes of one type of bacteria or a larger organism are combined with another type of bacteria.

Through this process, genes of plants, animals or even man could be grown in bacteria. And a living organism, such as a bacterium, could be given characteristics that it never had in its natural form.

Scientists at the Washington University and St. Louis University schools of medicine say that only a few researchers here will be directly affected by the guidelines, which were announced yesterday by Dr. Donald S. Fredrickson, director of the National Institutes of Health.

The guidelines supplant a less formal moratorium established about two years ago by scientists involved in such work, and are more stringent than recommendations circulated earlier this year by the National Institutes.

Prohibited under the guidelines are combining genes from certain dangerous disease organisms into others; transferring drug-resistant traits to microorganisms that could compromise the use of a drug to control disease, and forming recombinants from genes that can produce potent poisons.

For other experiments, graded safety precautions are described in detail, including descriptions of the kinds of organisms that can be used and the types of laboratories where the work may be done.

The studies believed to be the most hazardous may, under the guidelines, be done only in high security laboratories where the risk of research material escaping into the environment can be minimized.

Such a laboratory is in operation at St. Louis University's Institute for Molecular Virology. The laboratory was built about two years ago through a \$1,500,000 grant from the National Cancer Institute, says its director, Maurice Green. It contains complete filtering systems, artificially lowered air pressure, and sterilization equipment for supplies.

In a telephone interview yesterday, Green said that his work in the laboratory was directed toward determining the mechanism by which genes from certain viruses cause cells in small animals to become cancerous.

Green said the dangers addressed by the National Institutes' guidelines were not present in his lab, because it is not transplanting the viral genes into bacteria, but into cells from animals instead.

Green said he welcomed the guidelines, which he says will caution researchers of the potential dangers of their work. He said he was concerned by

the possibility that dangerous forms of common bacteria could escape from laboratories and contaminate the environment.

"Whether the possibility is real or not, nobody knows," Green said. "Nobody wants to take the chance."

Robert G. Roeder, a professor of biological chemistry at the Washington University School of Medicine, said in an interview yesterday that the physical safeguards in laboratories where controversial genetic research is taking place are not sufficient to prevent contamination.

"There's a legitimate worry," Roeder said, because researchers commonly produce genes in a type of bacteria (escherichia coli) "that is found in the human gut."

Roeder is experimenting with the production of recombinant DNA from the genes of amphibious animals. The research is considered harmless, he says, but he shares his colleague's concern over the dangers of the more controversial forms of genetic research.

Both Roeder and Green called for more widespread use of "biological safeguards," which do not rely on physically isolating the research material from the outside environment. For example, strains of bacteria that can only live under unusual conditions, such as high temperatures, can be used in the experiments.

Other scientists here said that caution should be a watchword.

"Nobody really wants to say we shouldn't do these experiments," said Sandra Schlessinger, a professor of microbiology at the Washington University School of Medicine. "Some are saying, though, that we should go more slowly."

"There's a growing awareness among scientists that there are hazards in the things they do," said Milton J. Schlessinger, her husband, who is also a professor of microbiology at the university.

In a letter published in the June 4 issue of Science magazine, the publication of the American Academy for the Advancement of Science, Erwin Chargaff of Columbia University wrote:

"There arises a general problem of the greatest significance, namely the awesome irreversibility of what is being contemplated. You can stop splitting the atom, you can stop visiting the moon, you can stop using aerosols, you can even decide not to kill entire populations by the use of a few bombs, but you cannot recall a new form of life."

Depending upon the genetic material used and the experimental objectives, the laboratories can range from ones similar to common college labs to others with elaborate systems of airlocks and filters resembling space capsules.

Fredrickson said the guidelines are effective immediately at NIH laboratories and those of its contractors and grantees. NIH currently supports about \$4 million worth of this research, he added.

The guidelines are not mandatory for research outside NIH, but Fredrickson said it is hoped all doing this type of work, including private industry, will comply voluntarily.

International health and scientific organizations have been awaiting the guidelines, said NIH officials, and several indicate they plan to adopt them in other countries.

The guidelines replace informal recommendations drawn up last year by an international group of scientists on how to conduct the research and on what work should not take place. Fredrickson said most scientists welcomed the guidelines.

"The greatest fear of the scientific community is that there is tremendous potential power in this technique and they want to be very conservative about using it," he said.

MESSANGER AND INQUIRER
Owensboro, Ky., 6/24/76

in the
nation

Genetic research precautions told

WASHINGTON (AP) — The National Institutes of Health (NIH) took steps Wednesday to prevent potential health hazards associated with a controversial kind of genetic research.

Dr. Donald S. Fredrickson said the NIH guidelines issued for "recombinant DNA" studies would help limit dangers of creating unknown diseases associated with the potentially beneficial research.

Fredrickson said the "generally conservative guidelines" would ban some potentially dangerous work and set up more rigid standards than presently used to reduce the risk of accidents that could release dangerous organisms into the environment.

DNA — deoxyribonucleic acid — is the chemical forming genes, the basic units of heredity. Recombinant DNA molecules result from recombining in a test tube DNA from different species of life.

Bacteria modified with recombinant DNA reproduce and have offspring with characteristics of the other species.

Supporters say the research has vast potential for scientific breakthroughs. For example, they see turning bacteria into little factories producing valuable proteins and hormones, such as insulin and blood clotting factor.

Critics say the research can create super disease organisms not found in nature that are immune to all known defenses and treatments.

The new guidelines ban research combining genes from certain risky disease organisms into others and prohibit transferring drug-resistant traits to microorganisms that could compromise use of a drug in controlling disease.

The guidelines go into great de-

tail describing four classes of laboratories in which the research can be done.

Depending upon the genetic material used and the experimental objectives, the laboratories can range from ones similar to common college labs to others with elaborate systems of airlocks and filters resembling space capsules.

Fredrickson said the guidelines are effective immediately at NIH laboratories and those of its contractors and grantees. NIH currently supports about \$4 million worth of this research, he added.

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HERALD-REPUBLIC
Yakima, Wash., 6/24/76

Hazards of genetic research watched

Some Research Forms Banned

Guidelines Issued on Life Creation

By Stuart Auerbach
Washington Post Staff Writer
After two years of intensive debate, the National Institutes of Health issued strict guidelines yesterday designed to allow scientists to create new forms of life without endangering the world.

These guidelines, which are expected to be adopted by other federal agencies and scientists across the globe, ban certain forms of research as too dangerous and set biological and physical safeguards for other experiments.

This new form of genetic

engineering, on the frontier of today's biological revolution, holds the promise of increasing the world's food supply and making scarce medicines readily available at a low price.

But the creation of new forms of life also raises the possibility of untold hazards—including creating more drug-resistant germs or increasing the incidence of cancer.

"You can conceive an endless list of possibilities of hazards," said Dr. Donald S. Fredrickson, director of NIH. "It is really the unknown that is the source of the fear, not specific and

certain hazards that could happen."

But the unknown hazards were enough to prompt scientists working in the field of recombinant DNA (deoxyribonucleic acid which determines inherited traits) to call in June, 1974, for a moratorium until guidelines could be drawn. Recombinant DNA is the combining of DNA molecules of different species to create new forms of life.

Fredrickson said the moratorium represented the first time in the history of science that researchers have voluntarily stepped back to make sure their ex-

periments would not cause greater harm than good.

The NIH guidelines, Fredrickson said, are stricter than ones drafted by an international committee of scientists meeting in March, 1975, at Asilomar, Calif., and by an NIH advisory committee.

For example, the new guidelines, which govern NIH-sponsored research, forbid letting any new forms of life into the atmosphere.

Also banned outright are experiments involving highly poisonous substances such as the virus that causes

See GENETICS, A15, Col. 2

JOURNAL
Providence, R.I., 6/24/76

Genetic research guidelines issued

NIH Issues Strict Guidelines on Life Creation

GENETICS, From A1

the deadly Lassa fever; the use of some viruses known to cause cancer; the formation of hybrids that contain the genes for poisons, including snake venom, or the transfer of drug-resistant traits to germs.

The NIH guidelines follow a general rule that the riskier the experiment, the more stringent the safeguards should be.

These include the use of supersafe labs with ventilation and sewerage systems that make sure contaminated air and wastes do not get outside. In such labs which can cost up to \$750,000, experiments contain chemicals manipulated through built-in heavy gloves.

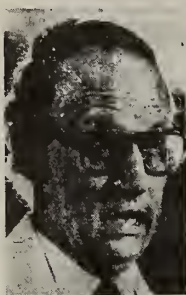
The guidelines require the use of "failsafe bugs" that

cannot exist outside of special laboratory conditions.

Recombinant DNA research, became possible within the past four years with the discovery that enzymes can chemically cleave the twisted strands of DNA, leaving them with sticky ends "that can be joined with other cut pieces of DNA."

THESE COMBINED STRANDS OF DNA—new forms of life not known in nature—can be transplanted into bacteria, where they will reproduce quickly.

Aside from the new knowledge it will provide about basic genetics, Fredrickson said the technique has practical benefits. It can, for example, allow the production of large amounts of rare hormones such as antihemophilic globulin, used to treat bleeding



DONALD S. FREDRICKSON.
... "an endless list"

diseases, or insulin, which is becoming rarer and is needed for diabetics.

Agricultural scientists are

hoping to graft on to other plants a gene that allows plants such as beans and alfalfa to take nitrogen from the air and make its own fertilizer.

Despite the safeguards proposed in the NIH guidelines, some scientists believe that any recombinant DNA research is too risky to be pursued. In Cambridge, Mass., for example, a group of Harvard and Massachusetts Institute of Technology researchers are fighting Harvard's plans to build a special lab for such experiments.

Fredrickson said NIH will file a statement by Sept. 1, on the environmental impact of the guidelines, which are effective immediately.

NIH spends about \$4 million to fund recombinant DNA research and has an-

other \$3.5 million available to improve labs to meet the new safety standards. In order to get this money, Fredrickson said, scientists must follow the guidelines.

The other major federal source of money for this research, the National Science Foundation, will recommend that its rule-making body, the National Science Board, adopt the NIH guidelines, foundation officials said.

Research

JOURNAL
Montgomery, Ala., 6/23/76

Genetic Research Guidelines Ban Some 'Dangerous' Experiments

TRIBUNE
Lewiston, Idaho, 6/24/76

New life

A guide to genetic testing

WASHINGTON — After two years of intensive debate, the National Institutes of Health issued strict guidelines Wednesday designed to allow scientists to create new forms of life without endangering the world.

These guidelines, which are expected to be adopted by other federal agencies and scientists across the globe, ban certain forms of research as too dangerous and set biological and physical safeguards for other experiments.

This new form of genetic engineering, on the frontier of today's biological revolution, holds the promise of increasing the

world's food supply and making scarce medicines readily available at a low price.

But the creation of new forms of life also raises the possibility of untold hazards—including creating more drug-resistant germs or increasing the incidence of cancer.

"You can conceive an endless list of possibilities of hazards," said Dr. Donald S. Fredrickson, director of NIH. "It is really the unknown that is the source of the fear, not specific and certain hazards that could happen."

But the unknown hazards were enough to prompt scientists working in the field of recombinant DNA (deoxyribonucleic acid, which

determines inherited traits) to call in June 1974 for a moratorium on research until guidelines could be drawn. Recombinant DNA is the combining of DNA molecules of different species to create new forms of life.

Fredrickson said the moratorium represented the first time in the history of science that researchers have voluntarily stepped back to make sure their experiments would not cause greater harm than good.

The NIH guidelines, Fredrickson said, are stricter than ones drafted by an international committee of scientists meeting in March 1975, at Asilomar, Calif., and by an NIH advisory committee.

For example, the new guidelines, which govern NIH-sponsored research, forbid letting any new forms of life into the atmosphere.

Also banned outright are experiments involving highly poisonous substances such as the virus that causes the deadly Lassa fever; the use of some viruses known to cause cancer; the formation of hybrids that contain the genes for poisons, including snake venom, or the transfer of drug-resistant traits to germs.

The NIH guidelines follow a general rule that the riskier the experiment, the more stringent the safeguards should be.

EVENING NEWS
Buffalo, N.Y., 6/23/76

NIH Issues Genetic Research Guidelines, Bar on Some Experiments

In Our Opinion

NIH Genetic Rules Were Needed Measure

The National Institutes of Health Wednesday issued a series of long-awaited guidelines for controversial types of genetic research, including outright bans on some particularly dangerous procedures.

Genetic research is a scientific field which has taken off in recent years and offers vast potential in the field of health, as well as in other areas. It involves altering the gene structure of organisms to give them characteristics they don't ordinarily have, or to remove undesirable characteristics.

The manipulation is made possible by new tools and methods which enable scientists to look into the genetic structure of a cell and alter the spiral helix of deoxyribonucleic acid — the chemical that builds genes.

A very simple example of what could be done through genetic manipulation would be something like determining the color of a newborn child's hair or eyes by altering the genes contained in the parents' sperm and egg.

While offering vast benefits, genetic research also holds the potential for ghastly horrors. It is entirely conceivable that by design or accident a virulent disease immune to any form of

treatment could be loosed upon the world.

It is to lessen such a likelihood that the NIH has issued its guidelines. The action came after scientists from all over the world expressed concern over the potential harm entailed in genetic research. Scientists were so concerned that, at the International meeting in 1975, they issued guidelines of their own.

The NIH guidelines are highly technical, but suffice it to say that they would outlaw completely certain types of highly dangerous experiments and restrict others to highly controlled environments that minimize the possibility of accidents.

Genes and the chemicals that make them up are the very building blocks of life itself. Their manipulation gives man an almost God-like potential to create new forms of life and to alter present forms into more desirable molds.

As with any endeavor that promises great good, there is also the potential for great evil. The NIH guidelines released Wednesday are a concrete way of minimizing the chance that the evil will escape. The guidelines were urgently needed and should now be stringently enforced.

POST

Denver, Colo., 6/23/76

'Rules Issued to Ban 'Too Dangerous' DNA Research

WASHINGTON —(AP)— The National Institutes of Health (NIH) issued long-awaited guidelines Wednesday for a controversial type of genetic research. They include a ban on some experiments considered too dangerous to perform.

The new rules for recombinant DNA are designed to control what some see as the almost God-like potential of science to create new forms of life.

CRITICS FEAR the research could create super disease organisms not found naturally and immune to all defenses against them. Supporters say the research has vast scientific potential. They envision turning bacteria into little generators of valuable proteins and hormones, such as insulin and blood clotting factor.

Dr. Donald S. Fredrickson, NIH director, said the guidelines are effective immediately at NIH laboratories and those of its contractors and grantees. NIH said the guidelines are more stringent than

proposals it circulated at the first of the year, and stricter than recommendations by an international meeting of scientists issued in 1975.

DNA—deoxyribonucleic acid — is the chemical that forms genes, the basic units of heredity. Recombinant DNA molecules result from recombining in a test

tube DNA from different species of life.

Genes for specific inherited traits taken from one species are combined with those of another, usually by removing a gene from a higher organism and putting it into bacteria. When the modified bacteria reproduces, the offspring contain characteristics of the new gene.

The new guidelines prohibit combining genes from certain risky disease organisms into others, transferring drug-resistant traits of microorganisms that could compromise use of a drug to control disease, and forming recombinants with genes that can synthesize potent poisons. The new directions also prohibit large-

scale experiments—those with more than 10 liters of culture—with recombinants known to make harmful products.

However, NIH said specific large-scale experiments that could directly benefit society may be allowed under special conditions with specific NIH approval.

The guidelines list four classes of

laboratories in which the research can be done. Depending on the experiment and genetic material involved, the laboratories range from common college labs to ones with elaborate systems of airlocks and filters resembling space capsules.

The Recombinant Advisory Committee at NIH, which helped put together the guidelines after conducting public hearings, will remain in existence to interpret the guidelines and answer questions that may arise, Fredrickson said.

IT IS UNKNOWN how much recombinant DNA research is going on, Fredrickson said, "but there are indications that industry already is involved in this research to a wide extent."

Fredrickson said private industry isn't required to report what kind of research it is doing, nor are the guidelines mandatory for research outside NIH. "It is our hope that they will be effective as guidelines with voluntary compliance," he said.

COURIER

Evansville, Ind., 6/28/76

Cutting, splitting genes spurs scientific debate

By NICHOLAS WADE

(A reporter for Science Magazine)

L.A. Times Washington Post Service

In the next few weeks a new and adventurous technology will be unleashed on the world by way of guidelines issued at the command of the head of a minor federal agency.

The technology is based on a method for creating new forms of life, a prerogative hitherto reserved for evolution. Whereas new organisms arise in nature through the slow and haphazard reshuffling of genes, the method now available enables the scientist to manipulate the genetic material directly by a technique of cutting and splicing.

This presents benefits and risks on a scale probably comparable with those of harnessing the atom. The benefits, which are easily visible and near at hand, appear at present to outweigh the more nebulous and seemingly distant hazards.

It would be untrue to say that the scientific community is divided on the merits of pushing ahead with the development of the technology. Most scientists believe that the dangers are negligible or can be contained.

In fact, the present plans to proceed, in the form of guidelines to be issued soon by the director of the National Institutes of Health, have only two serious critics.

YET THE CRITICS happen to be two of the most distinguished of all the scientists involved in the decision-making process.

Also, unlike many of their colleagues, they have no personal interest in using the new techniques in their own research.

Robert Sinsheimer, a member of the National Academy of Sciences and chairman of the biology division at Cal-Tech, commented recently of the proposed NIH guidelines:

"Obviously neither I nor anyone else can say that if the present committee guidelines are adopted, disaster will ensue. I will say, though, that in my judgment, if the guidelines are adopted and nothing untoward happens, we will owe this success far more to good fortune than to human wisdom."

And Erwin Chargaff of Columbia University, who holds the National Medal of Science, points out that new forms of life, once created, cannot be recalled from nature.

"Have we the right to counteract, irreversibly, the evolutionary wisdom of millions of years, in order to satisfy the ambition and curiosity of a few scientists?" he asks in a letter in Science.

To do so, he believes, is a folly and a crime, an attack on the biosphere "so unthinkable to previous generations, that I could only wish that mine had not been guilty of it."

WHAT HAS MADE the new technology possible is not so much man's cleverness as the accidental discovery of something in nature, a class of bacterial enzymes endowed with quite unexpected properties. The enzymes constitute a molecular-level scissors-and-paste kit for cutting and splicing the genetic material with undreamed of precision.

The technique of assembling new genetic messages—known as "recombinant DNA" molecules—is a powerful research tool that will doubtless bring its earliest users a crop of Nobel prizes and the like. It also offers a cornucopia of practical applications in the form of custom designing organisms for use in industry, agriculture and medicine.

Scientists first drew attention to the hazards two years ago when a group of American researchers called for a voluntary moratorium until appropriate safety conditions could be established.

Scientists throughout the world have observed the moratorium, and will probably follow closely the NIH guidelines that will bring it to an end.

AND YET, despite the two years of self-restraint, the guidelines about to be issued will allow almost all but the most wildly dangerous experiments to proceed under strict but not grossly inconvenient safety conditions.

The public has had little effective opportunity to share in the decision-making process, and the fundamental objections of inside critics such as Sinsheimer and Chargaff have been ignored without being specifically rebutted.

What are the dangers of the new technique and the technology that will sprout up from it? First, the new forms of life can be expected to escape quite regularly, particularly from the less meticulous laboratories.

Even the most careful scientists become exposed to the organisms they work with, and the best available containment methods used in the Army's biological warfare laboratories at Fort Dietrich, for example, did not prevent 423 cases of infection and 3 deaths over a period of 25 years.

Escape is made more certain by the circumstance that the bacterium to be used as host for many of the new life forms is *Escherichia coli*, a common inhabitant of the human gut and nose.

If a human pandemic or some other "worst case" accident occurs, future historians will never be able to understand why of all the bacterial species at our disposal, we made the most reckless possible choice.

MOST OF THE NEW life forms will perish outside the laboratory, unless deliberately designed to survive. But some may find suitable niches, in our crop plants, in our domestic animals, or in human populations. At worst, the consequences could range from mass infection to the virtual eradication of a species.

People worry about the proliferation of nuclear technology to more than a handful of countries. The "recombinant DNA" technique puts what will one day become an almost equally awesome technology into the hands of every biological researcher with access to a modern laboratory.

Sooner or later, one of these researchers may try to put the technology to evil use. But more to be feared is the do-gooder who attempts to take some unilateral action for what he conceives to be the benefit of mankind.

ENQUIRER

Cincinnati, Ohio, 6/24/76

NIH Issues Guidelines On Controversial Genetic Experiments



Copy of letter to professional societies

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

June 30, 1976

See attached list of addressees

Gentlemen:

Enclosed is a copy of the NIH guidelines on recombinant DNA research and the decision paper that accompanies them.

These guidelines will govern research at laboratories of NIH and at those of its grantees and contractors. NIH has also undertaken an "environmental impact assessment," in accordance with the National Environmental Policy Act of 1969. The purpose of this assessment is to review the environmental effects, if any, of research that may be conducted under the guidelines.

The NIH guidelines establish carefully controlled conditions for the conduct of experiments involving the production of recombinant DNA molecules and their insertion into organisms such as bacteria. These guidelines replace the recommendations contained in the 1975 Summary Statement of the Asilomar Conference on Recombinant DNA Molecules. The latter would have permitted research under less strict conditions.

Within the month, NIH will publish a report containing the full transcript of a public hearing held at NIH on February 9-10, 1976; the statements filed by public and scientific witnesses at that hearing; and the correspondence addressed to me on this matter. My decision, which is based on that record, examines each of the substantive issues presented with an explanation of my decision on each issue. These guidelines contain a number of revisions based on that analysis. On the basis of continuing discussions with other organizations that are conducting, monitoring, or supporting this type of research in this country and abroad, we believe these guidelines will be adopted by other laboratories throughout the United States and that they will provide a basis for development of similar guidelines in foreign countries.

Much of the public commentary directed to me on the proposed guidelines focused on the expressed concern that there should be guidelines for recombinant DNA research not supported or conducted by NIH. There was general agreement that NIH should undertake to disseminate information on

Page 2

the guidelines as widely as possible to all who conduct or support recombinant research. I would especially welcome consideration of these guidelines by your organization and your membership. Also, in view of the stated public concern for uniformity in the conduct of this research, I would be especially interested in your society's potential support and, where appropriate, endorsement of the NIH guidelines.

If you have any questions or comments on the guidelines or the issues raised in the decision paper, I would be pleased to respond to them. Thank you.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Enclosures

List of professional societies that received letter of June 30, 1976

Academy of Pharmaceutical Sciences
2215 Constitution Avenue, N.W.
Washington, D.C. 20037

American Academy of Arts and Sciences
165 Allandale Street
Jamaica Plain Station
Boston, Massachusetts 02130

American Association for Cancer Research
Institute for Cancer Research
7701 Burholme Avenue
Philadelphia, Pennsylvania 19111

American Association for Maternal
and Child Health
116 South Michigan Avenue, Suite 806
Chicago, Illinois 60603

American Association for the
Advancement of Science
1515 Massachusetts Avenue, N.W.
Washington, D.C. 20005

American Association of Anatomists
University of Arkansas Medical Center
Little Rock, Arkansas 72201

American Association of Immunologists
9650 Rockville Pike
Bethesda, Maryland 20014

American Association of Pathologists
and Bacteriologists
9650 Rockville Pike
Bethesda, Maryland 20014

American Association of Physicists
in Medicine
335 East 45th Street
New York, New York 10017

American Cancer Society
219 East 42nd Street
New York, New York 10017

American Chemical Society
1155 Sixteenth Street, N.W.
Washington, D.C. 20036

American College of Physicians
4200 Pine Street
Philadelphia, Pennsylvania 19104

American Federation for Clinical
Research
6900 Grove Road
Thorofare, New Jersey 08086

American Genetic Association
1028 Connecticut Avenue, N.W.
Washington, D.C. 20036

American Institute of Biological
Sciences
3900 Wisconsin Avenue, N.W.
Washington, D.C. 20016

American Medical Association
535 N. Dearborn Street
Chicago, Illinois 60610

American Pharmaceutical Association
2215 Constitution Avenue, N.W.
Washington, D.C. 20037

American Physiological Society
9650 Rockville Pike
Bethesda, Maryland 20014

American Society for Cell Biology
Department of Anatomy
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461

American Society for Clinical
Investigation
c/o Paul Calabresi, M.D.
Roger Williams General Hospital
825 Chalkston Avenue
Providence, Rhode Island 02908

American Society for Microbiology
1913 I Street, N.W.
Washington, D.C. 20006

American Society for Pharmacology and
Experimental Therapeutics
9650 Rockville Pike
Bethesda, Maryland 20014

American Society of Biological Chemists
9650 Rockville Pike
Bethesda, Maryland 20014

American Society of Cytology
7112 Lincoln Drive
Philadelphia, Pennsylvania 19119

American Society of Human Genetics
Department of Medical Genetics
Indiana University Medical Center
1100 W. Michigan Street
Indianapolis, Indiana 46202

American Society of Internal Medicine
525 Hearst Building
Third at Market
San Francisco, California 94103

American Society of Zoologists
Box 2739
California Lutheran College
Thousand Oaks, California 91360

Association of Academies of Science
111 Columbia Drive
Oak Ridge, Tennessee 37830

Association of American Medical Colleges
One Dupont Circle
Washington, D.C. 20036

Association of American Physicians
c/o John A. Oates, M.D.
Vanderbilt University School of Medicine
Nashville, Tennessee 37232

Biophysical Society
c/o Dr. Margaret O. Dayhoff
National Biomedical Research Foundation
Georgetown University Medical Center
3900 Reservoir Road, N.W.
Washington, D.C. 20007

Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014

Genetics Society of America, Inc.
c/o Dr. Margery Shaw
Department of Zoology
University of Texas
Austin, Texas 78712

National Genetics Foundation
250 West 57th Street
New York, New York 10019

National Society for Medical Research
1330 Massachusetts Avenue, N.W.
Suite 103
Washington, D.C. 20005

New York Academy of Medicine
2 East 103rd Street
New York, New York 10029

New York Academy of Sciences
2 East 63rd Street
New York, New York 10021

Society for Developmental Biology
Box 502
Kalamazoo, Michigan 49005

Society for Experimental Biology
and Medicine
630 West 168th Street
New York, New York 10032

Society for Pediatric Research
1621 E. 119th Street
Los Angeles, California 90059

Society of General Physiologists
c/o Charles Edwards, Sec.
Marine Biological Laboratory
Woods Hole, Massachusetts 02543



Letter to editors of scientific journals

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

July 1, 1976

Dear

Enclosed is a copy of the National Institutes of Health guidelines on recombinant DNA research and the decision paper that accompanies them. These guidelines will govern research at laboratories of NIH and at those of its grantees and contractors.

NIH has also undertaken an "environmental impact assessment," in accordance with the National Environmental Policy Act of 1969. The purpose of this assessment is to review the environmental effects, if any, of research that may be conducted under the guidelines.

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Within the month, NIH will have in press a public record containing the NIH guidelines, a report on my decision to release them, the full transcript of a public hearing held at NIH on February 9-10, the statements filed by public and scientific witnesses at that hearing, and the correspondence addressed to me on this matter. The guidelines contain a number of revisions based on analysis of this public record. The report on my decision to release the guidelines examines each of the substantive issues presented and explains these revisions. On the basis of continuing discussions with other organizations that are conducting, monitoring, or supporting recombinant DNA research, we believe these guidelines will be adopted by other laboratories throughout the United States and will provide a basis for development of similar guidelines in foreign countries.

Much of the public commentary directed to me on the proposed guidelines expressed concern that there should also be guidelines for recombinant DNA research not supported or conducted by NIH. There was general agreement that NIH should undertake to disseminate information on the guidelines as widely as possible to all who conduct or support such research.

Quite clearly there is public concern for uniformity in the conduct of recombinant DNA research and for adherence by all to a uniform standard. Hence, I ask you to consider requesting investigators to include a description of the physical and biological containment procedures used in any such research they report in your publication. You will note that this recommendation is included in the guidelines on page 3. The recommendation comes from those in the scientific community who were instrumental in organizing the Asilomar meeting and calling on NIH to undertake the responsibility for developing these guidelines. It is also a recommendation of the NIH Recombinant DNA Advisory Committee that proposed the guidelines. I would very much appreciate hearing your views on this matter. Your support in this regard will be extremely helpful in assuring the public and others concerned that the scientific community recognizes its responsibility in this important research area.

If you have any questions or comments on the guidelines or the issues raised in the decision paper, I would be pleased to respond to them. Thank you.

Sincerely yours,

/s/

Donald S. Fredrickson, M.D.
Director

Enclosures

LIST OF SCIENTIFIC JOURNALS AND EDITORS THAT RECEIVED THE GUIDELINES

Edward D. Frolich, Editor
The Journal of Laboratory and
Clinical Medicine

Henry G. Schwartz, Editor
Journal of Neurosurgery

Dr. Irwin M. Freedberg, Editor
The Journal of Investigative
Dermatology

Waldo E. Nelson, M.D.
The Journal of Pediatrics

Dr. Ernst R. Jaffe
Blood: The Journal of the
American Society of Hematology

Herbert Sloan, M.D.
The Annals of Thoracic Surgery

Editor
Arthritis and Rheumatism

Dr. Hugh J. Jewett, Editor
The Journal of Urology

Richard F. Mattingly, M.D., Editor
Obstetrics and Gynecology

Editor
Physical Therapy

Editor
Postgraduate Medicine

Dr. George E. Burch, Editor
American Heart Journal

Nicholas M. Greene
Anesthesiology

Dr. Jean D. Wilson, Editor
The Journal of Clinical Investigation

Editorial Office
Journal of Medical Education

Editorial Department
Journal of Oral Surgery

Dr. Brian Blades, Editor
The Journal of Thoracic and
Cardiovascular Surgery

John S. Chapman, M.D.
Archives of Environmental Health

Editor
Archives of Physical Medicine and
Rehabilitation

John H. Davis, M.D., Editor
The Journal of Trauma

Dr. A. McGehee, Editor
Medicine

Dr. Jerold F. Lucey, Editor
Pediatrics

Frank McDowell, M.D., Editor
Plastic and Reconstructive Surgery

Frank W. Newll, M.D.
American Journal of Ophthalmology

Paul C. Beaver, Editor
Tropical Medicine and Hygiene

Editorial Office
American Journal of Surgery

Editor
The American Journal of Psychiatry

Dr. Melvin M. Figley, Editor
American Journal of Roentgenology

Thomas D. Kinney, M.D., Editor
The American Journal of Pathology

David C. Sabiston, Jr., M.D.
Annals of Surgery

Ben H. Senturia, M.D., Editor
Annals of Otolaryngology, Rhinology &
Laryngology

H.D. Bouman, M.D., Editor
American Journal of Physical
Medicine

Editor
Disease-A-Month

Editor
The Medical Letter, Inc.

John H. Epstein, M.D., Chief
Editor
Archives of Dermatology

Daniel X. Freedman, M.D.
Archives of General Psychiatry

Editorial/Production Offices
American Family Physician

Valeda Slade, Managing Editor
Inquiry

Edmund H. Sonnenblick, M.D.,
Editor
Progress in Cardiovascular
Diseases

The Editor
The Journal of Infectious Diseases

Editor
The American Journal of Clinical
Nutrition

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